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OCCURRENCE, CONTROL, AND PERSISTENCE OF THE CYANOBACTERIAL TOXIN, MICROCYSTIN-LR

BY

ANGELINE KA-YUK LAM

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF BIOLOGICAL SCIENCES

EDMONTON, ALBERTA
FALL 1994

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The undersigned certify that they have read, and recommend to the Faculty of Graduates Studies and Research, for acceptance, a thesis entitled **OCCURRENCE, CONTROL, AND PERSISTENCE OF THE CYANOBACTERIAL TOXIN, MICROCYSTIN-LR** submitted by **ANGELINE KA-YUK LAM** in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**.

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Abstract

Freshwater cyanobacteria are known to produce a family of at least 50 cyclic hepatotoxins called microcystin (MCYST). Microcystin-LR (MCLR), the most commonly reported potent MCYST, was frequently found in three drinking water supply lakes (Little Beaver, Driedmeat, and Coal) in Alberta from 1991-1993. Two genera of cyanobacteria found in these lakes, *Microcystis* and *Anabaena*, were reported to produce MCLR. However, MCLR concentration, expressed as μg per g phytoplankton biomass (dry weight), was correlated with the abundance of *Microcystis* and not with *Anabaena* (r=0.63 and -0.13, n=135, P<0.0001 and =0.13, respectively). Thus, *Microcystis* was probably the major MCLR-producer in these lakes.

The MCLR concentrations were variable within each lake and among years (ranged from ≤ 1 $\mu g \cdot g^{-1}$ to 1300 $\mu g \cdot g^{-1}$ biomass; by high performance liquid chromatography), reflecting the variability in both the abundance of *Microcystis* and the production of MCLR by *Microcystis* cells within lakes and over time. Peak biomass of *Microcystis* was found in a wide range of water temperatures (12-20 °C). The abundance of *Microcystis* was positively correlated with total phosphorus concentration and pH, and was negatively correlated with nitrogen to phosphorus ratio and Secchi depth. The notion that blooms may be toxic one day and not the next was not supported in this study. When all data in these lakes over the three years were pooled, over 40% of the variability in MCLR could be explained based on the abundance of *Microcystis aeruginosa*. The production of MCLR by *Microcystis* cells apparently consisted of 3 phases over the lifetime of a bloom: Phase 1, decline in intracellular MCLR; Phase 2, stable intracellular MCLR; and Phase 3, increase in intracellular MCLR; thus, direct monitoring of MCLR concentrations in drinking water lakes is needed for health risk assessments.

The effects of two alternative approaches for chemical removal of phytoplankton was examined first in a laboratory setting with freshly collected phytoplankton (1 $g \cdot L^{-1}$ phytoplankton biomass at 182 to 837 μg MCLR·g^{-1} biomass; dry weight) and then in a lake setting (2 $mg \cdot L^{-1}$ phytoplankton biomass at 0.48 to 1.4 μg MCYST·g^{-1} biomass) with closed-bottom limnocorrals. Six chemical treatments were categorized into two groups:

Group 1 chemicals disrupt cell functions and induce cell lysis; they include Reglone A, chlorine, potassium permanganate, and Simazine; and Group 2 chemicals precipitate (or coagulate) phytoplankton cells but leave them essentially intact; they include lime and alum. Results from both studies were consistent in that treatment with Reglone A removed phytoplankton (primarily cyanobacteria) blooms with a concomitant increase in dissolved MCYST (exo-MCYST) concentration in the surrounding water. In contrast, the lime and/or alum treatments (when lake water pH was maintained between 7 and 10) removed blooms without a concomitant increase in exo-MCYST concentrations in the surrounding water. The extent of cell damage, as examined qualitatively by scanning electron microscopy and transmission electron microscopy, appeared to correspond with the exo-MCLR concentration in chemically-treated water. The estimated half-life of released MCLR from concentrated cyanobacterial blooms in the laboratory setting ranged from 0.5 (±0.1) to 1.6 (±0.0) d. However, no apparent exo-MCYST degradation occurred for ≥ 5 d in the closed-bottom limnocorrals. The discrepancy between the persistence of MCLR in lake water in the laboratory setting and in lake setting was likely caused by higher biomass and condition of cells in the laboratory setting.

Two primary pathways were considered as natural routes of MCLR removal: accumulation in bottom sediment and biotransformation. Controlled experiments based on the presence or absence of a sorbent medium (lake sediment) and bacteria (wastewater effluent) were conducted; MCLR concentrations were monitored over time. In the sorption experiments, MCLR did not partition onto lake sediments ($P>0.99$); thus, the decomposition of exo-MCLR in lakes is likely to occur via biotransformation and the closed-bottom limnocorrals likely mimicked lake processes fairly accurately with respect to microcystin dynamics. In the biotransformation experiments however, MCLR was susceptible to biotransformation only after a 10-d lag period. The transformed product(s) of MCLR, unlike the parent toxin, did not inhibit protein phosphatase activity. Based on first-order kinetics, the half-life of MCLR ranged from 0.2 to 3.6 d when the microbial population was in the order of 10^5 CFU·mL^{-1} using Plate Count Agar. Thus, in a lake, exo-MCYST likely has either a half-life or a lag period of biotransformation of ≥ 5d.

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List of Nomenclature or Abbreviations

A	=	Anabaena
Adda	=	3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl-deca-4,6-dienoic acid
Alum	=	$(Al_2SO_4)_3 \cdot 14H_2O$
Aph	=	Aphanizomenon
C	=	carbon
$CaCO_3$	=	calcium carbonate
Chl*a*	=	chlorophyll *a*
Chlorination	=	NaOCl or sodium hypochlorite
CO_3^{2-}	=	carbonate
$CuSO_4$	=	copper sulfate
DIN	=	total inorganic nitrogen
DOC	=	dissolved organic carbon
endo	=	intracellular
exo	=	extracellular or dissolved
Glu	=	D-glutamic acid
HPLC	=	high liquid performance chromatography
in situ	=	in place
i.p.	=	intraperitoneal injection: within the peritoneal cavity of the abdoman
K^+	=	potassium
$KMnO_4$	=	potassium permanganate
LD_{50}	=	lethal dosages of 50% of the population
lime	=	$Ca(OH)_2$
M	=	Microcystis
MAsp	=	erythro-β-methylaspartic
MCYST	=	total microcystins
MCLR	=	microcystin-LR
MCRR	=	microcystin-RR
Mdha	=	N-methyldehydroalanine
N	=	nitrogen
NH_4^+	=	ammonium
NO_3^-	=	nitrite+nitrate
P	=	phosphorus
PP	=	protein phosphatase
Reglone A	=	diquat or 1,1-ethylene-2,2-dipyridilium dibromide
SEM	=	scanning electron microscopy
Simazine	=	2-chloro-4,6-bis(ethylamino)-s-triazine
TEM	=	transmission electron microscopy
TN	=	total nitrogen
TP	=	total phosphorus
TDP	=	total dissolved phosphorus
WTemp	=	water temperature

I. General introduction: Background of microcystin-LR and scope of this study

Toxic cyanobacterial blooms have aroused worldwide attention since Francis (1878) first reported their occurrence in Australia. Since then, poisonings from cyanobacterial blooms have been reported in several countries (Falconer et al. 1983; Hawkin et al. 1985; Carmichael and Falconer 1993). In Canada, the first report on cyanobacterial poisoning was by Howard and Berry (1933). Many of the cyanobacterial blooms responsible for poisoning are dominated by one or more of the following genera of cyanobacteria (commonly referred to as blue-green algae): *Anabaena* spp., *Aphanizomenon* spp., *Microcystis* spp., and *Oscillatoria* spp. In a recent Alberta survey, toxic cyanobacterial blooms were detected only when *Microcystis aeruginosa* is present (Kotak et al. 1993).

M. aeruginosa was first reported to be linked with livestock deaths by Steyn (1945). However the study of toxins was hindered until Hughes et al. (1955, 1958) successfully isolated and cultured *M. aeruginosa*. The toxins produced by *M. aeruginosa* were named microcystins by Konst et al. (1965); the naming of each individual toxin was later systematised by Carmichael et al. (1988). Since then, knowledge concerning the isolation, identification, and specific pathological effects of the cyanobacterial toxins has grown enormously. This is because blooms of cyanobacteria are common in eutrophic (highly productive) lakes. Blooms produce potent toxins, and in western Canada, many prairie communities depend upon eutrophic lakes as their only drinking water supply. However, it is still not possible to predict when toxic blooms will occur.

Microcystis spp. produce a family of over 50 different microcystins. The basic structure of microcystins consists of five largely invariable amino acids (D-alanine, erythro-β-methylaspartic (MAsp), D-glutamic acid (Glu), N-methyldehydro-alanine (Mdha), and 3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl-deca-4,6-dienoic acid (Adda)), and two variable L-amino acids (Fig. 1). The most commonly reported form of microcystin has been microcystin-LR, which has an intraperitoneal injection LD_{50} of 50 $\mu g \cdot kg^{-1}$ in mice and an oral LD_{50} of 3100 $\mu g \cdot kg^{-1}$ in mice (based on Falconer et al. 1988). Prior to the initiation of this study, the fate of microcysitn-LR in aquatic environments has not been investigated. Chemicals such as copper sulfate, Reglone A, lime, and alum have

been commonly used to control cyanobacterial blooms without taking into account the potential for release of microcystins into the water. Reports on the outbreak of human hepatoenteritis in Australia in 1979 (Falconer et al. 1983), after the application of copper sulfate to a drinking water supply, suggested a need to better understand the effect of chemicals on toxin release from *M. aeruginosa*. The apparent association between cyanobacterial blooms and their toxins in eutrophic lakes, and the persistence of microcystins after traditional drinking water treatments (Hoffman 1976; Keifola et al. 1988; Himberg et al. 1989; Falconer et al. 1989), indicate the need for studies on the stability of microcystins in freshwater.

My research focused on the temporal dynamics, control, and stability of microcystin-LR in the aquatic environment, including: 1) succession of *Microcystis* blooms and periodicity of microcystin-LR in three shallow eutrophic hardwater lakes (CHAPTER II); 2) effect of up to six chemical treatments on release of toxin from *M. aeruginosa* in both laboratory (CHAPTER III) and lake (CHAPTER IV) setting; and 3) persistence of microcystin-LR with emphasis on accumulation in sediment by sorption (CHAPTER IV) and biotransformation (CHAPTER V).

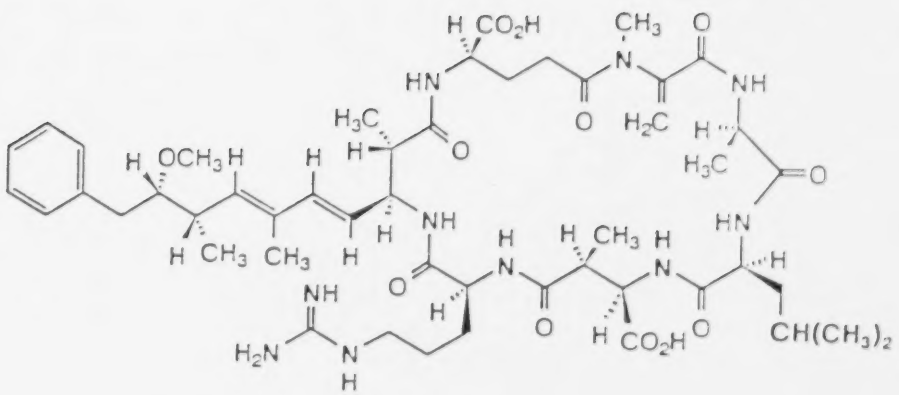

Fig. 1. Structure of microcystin-LR.

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II. Periodicity of *Microcystis* "within" blooms of cyanobacteria and intracellular microcystin-LR concentrations in three shallow eutrophic hardwater lakes[1, 2]

Introduction

Cyanobacteria are prokaryotic cells with gas vacuoles that allow them to float to the surface of stagnant lake water. Cyanobacteria are commonly found in eutrophic to hypereutrophic lakes. When cyanobacteria abundance is high ("bloom"), the lake appears to have a "green scum" on the surface, which spoils recreational (e.g., swimming and boating) and commercial (e.g., fishing) aspects of the lake. In addition, cyanobacterial blooms are often associated with taste and odour problems in drinking water, and are responsible for mechanical difficulties in water treatment plants, including shortened filter runs, clogged intake screens, slime layers on walls of filters and settling basins, and sludge deposits in settling basins.

Recent publications have shown that the occurrence of particular cyanobacterial toxins in lake water are correlated with certain genera of cyanobacteria. The two types of cyanobacterial biotoxins and associated cyanobacteria genera are: alkaloid neurotoxins (*Anabaena* (*Ana.*), *Aphanizomenon* (*Aph.*), and *Gomphosphaeria*) and cyclic hepatotoxins (*Microcystis*, *Anabaena*, and *Oscillatoria*). In fresh water, anatoxin-a (Antx-*a*) is the most commonly reported neurotoxin and microcystin-LR (MCLR; Fig. 1) is the most commonly reported hepatotoxin. Although Antx-*a* is more toxic than MCLR (LD_{50} of 20 and 50 $\mu g \cdot kg^{-1}$ in mice by intraperitoneal injection, respectively; Carmichael 1988), the latter is more stable and is more of a public health concern in drinking water supplies (Falconer et al. 1983; Meyer 1987; Marshall 1991; Carmichael and Falconer 1993). To understand the dynamics of MCLR in drinking water supplies, it is necessary to examine the characteristics or the seasonal succession of *Microcystis* blooms in eutrophic lakes.

Many studies have focused on what conditions allow cyanobacteria to dominate phytoplankton blooms. High water temperatures (Fogg et al. 1973; McQueen and Lean 1987), low inorganic carbon availability (Paerl and Ustach 1982; Shapiro 1973, 1984),

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1 A portion of data in this chapter has been submitted for publication. B. G. Kotak, A. K.-Y. Lam, E. E. Prepas, S. L. Kenefick, and S. E. Hrudey. *J. Phycol.*

2 A version of this chapter will be submitted for publication. A. K.-Y. Lam, B. G. Kotak, and E. E. Prepas. *Hydrobiologia*

low light availability (Zevenboom and Mur 1980), low total nitrogen to phosphorus ratios (TN:TP; Schindler 1977; Smith 1983), and high total phosphorus concentrations (Trimbee and Prepas 1987) are among the conditions that have been indicated to favour the growth of cyanobacteria. However, only a few studies have investigated cyanobacterial species succession within a bloom.

Hammer (1964) claimed that water temperature was a trigger for the appearance of *Ana. flos-aquae* (10-15 °C), *Aph. flos-aquae* (>20 °C) and *M. aeruginosa* (17-18 °C) and for the sequence of appearance of these species in cyanobacterial blooms in Saskatchewan lakes. These results were modified by Hammer (1965) and indicated that the log growth of *Aph. flos-aquae* occurs in the range of 17-19 °C. Further study by Hammer (1969) showed that the abundance of *Aph. flos-aquae* and *M. aeruginosa* in Saskatchewan lakes, but not of *Ana. flos-aquae*, were positively related to water temperature. Thus, it appeared that water temperatures may play a role in determining the abundance of a particular species in a bloom.

Smith et al. (1987) developed a model to predict the intensity of blooms of a single cyanobacterial species based on 14 stations in four Swedish lakes (Vänern, Vättern, Mälaren, and Hjälmaren). Their model suggests that the abundance of *M. aeruginosa* can be predicted by TP concentrations only, *Ana. flos-aquae* by TP, total nitrogen (TN), and mean water depth, and *Aph. flos-aquae* by TP, water temperature and carbon dioxide concentration. The model of Smith et al. (1987) was based on only four Swedish lakes, the applicability of their model to other regions may be limited.

The periodicity of *M. aeruginosa* in Lake Kasumigaura, Japan, has been studied extensively. Takahashi et al. (1981) showed that *M. aeruginosa* was strongly negatively correlated with ammonium concentration, and was positively correlated with water temperature for blooms occurring in July and August. Laboratory culture experiments with *M. aeruginosa* isolated from Lake Kasumigaura suggested that there was a substantial increase in *M. aeruginosa* growth when water temperature was <25 °C (Komatsu 1980). A recent study in Lake Kasumigaura again showed that *M. aeruginosa* was correlated with water temperature, pH, and also total and dissolved chemical oxygen demand (Ohkubo et al. 1993). To date, studies on the characteristics of individual cyanobacterial species in other lakes are rare but essential for understanding the occurrence and variability of cyanobacterial hepatotoxins in lakes.

This phytoplankton study was part of a program on cyanobacterial toxins in three eutrophic and hypereutrophic hardwater lakes in Alberta. From 1991 to 1993, cyanobacterial blooms were common in these lakes in July and August and sometimes into the fall. The dominant cyanobacterial species in a bloom in a particular lake was not

predictable. This paper describes the seasonal periodicity of phytoplankton communities in the three study lakes, with emphasis on *M. aeruginosa* and the associated cyanobacterial toxin MCLR, and seasonal variation of abiotic and biotic factors which may affect *M. aeruginosa* abundance and periodicity.

Materials and Methods

Three eutrophic hardwater lakes (Little Beaver, Driedmeat, and Coal) were selected as study sites on the basis of their repeatedly high biomass of cyanobacteria and concern about community health, as they all serve as municipal drinking water supplies. These lakes are located in the Battle River drainage basin in western Canada, have similar morphometry (Table 1; data from Mitchell and Prepas 1990; Kotak et al. submitted), and range in salinity from fresh (Coal and Driedmeat lakes) to moderately saline (Little Beaver Lake). In addition to being a drinking water supply lake for the nearby community of Camrose, Driedmeat Lake receives effluent from the wastewater treatment plant.

Water samples were collected from June to July 1991, May to October 1992, and May to August 1993 for chemical analyses and phytoplankton enumeration. Water samples were collected every second week in spring and early summer (May to June) and weekly thereafter (July to September). On each sampling trip, water temperature was measured *in situ* with a YSI model 51 O_2/temperature meter, pH was measured with a Fisher model 3D field pH meter, and transparency was estimated with a Secchi disk. In 1991, surface water was collected by immersing a 10-cm tall bottle vertically into the water column. In 1992-93 composite (4 sites), integrated water samples (from a depth of 1 m for Little Beaver Lake and 2 m for Driedmeat and Coal lakes to the surface) were collected with a Tygon tube (I.D. = 1.6 cm) fitted with a one-way foot valve. Unfiltered water samples were analyzed for TP, ammonium (NH_4^+), carbonate (CO_3^{2-}), and bicarbonate (HCO_3^-). Water samples filtered through 0.45-µm HAWP Millipore membrane filters, were used for total dissolved phosphorus (TDP) and nitrate+nitrite (NO_3^-) analyses. TP and TDP were analysed according to the modified potassium persulfate method (Prepas and Rigler 1982). NO_3^- and NH_4^+ concentrations were determined as described in Stainton et al. (1977), and HCO_3^- and CO_3^{2-} were analysed by sulfuric acid titration (to pH 4.5 and 8.3, respectively). In 1993, unfiltered water samples were also analysed for total Kjeldahl nitrogen (TKN; after D'Elia et al. 1977); total nitrogen is the sum of NO_3^- and TKN. Chlorophyll *a* (Chl*a*) was determined by the ethanol extraction technique described in Bergmann and Peters (1980). Water samples for phytoplankton enumeration were preserved with Lugol's

solution; phytoplankton counts were performed with a Carl Zeiss Sedival Hydrobiological Inverted Microscope with the sedimentation method described by Utermöhl (1958). Depending on the relative abundance of the phytoplankton, evaluations were made by filling a counting chamber 1 to 3 times with either a 0.75- or a 2.1-mL sample and evaluating 20 fields in each aliquot.

In addition to water samples, phytoplankton were collected for toxin analysis with a conical 64-µm mesh net (29-cm internal diameter by 90-cm long, fitted with a brass plankton bucket) which was either skimmed across the surface of lakes (in 1991) or hauled vertically (in 1992 and 1993) through the water column through the same depths as for collection of water for water quality analyses. To examine the periodicity of intracellular MCLR concentration, chl*a* was converted to dry weight. MCLR concentration in µg per gram dry weight of phytoplankton was then converted to pg MCLR per liter lake water, and finally to pg per *Microcystis* cells using the following equations.

Assuming that chl*a* constitutes, on average, 1.5% ash free dry weight of phytoplankton organic matter (APHA et al. 1989),

$$\text{phytoplankton biomass } (\mu g \cdot L^{-1}; \text{drywt.}) = 67 \times [\text{chl}a\ (\mu g \cdot L^{-1})]. \quad (1)$$

The MCLR concentration is converted from $\mu g \cdot g^{-1}$ to $pg \cdot L^{-1}$ using the equation,

$$\text{MCLR } (pg \cdot L^{-1}) = \{[\mu g \text{ MCLR per g biomass } (\mu g \cdot g^{-1})] \times 10^{6}\ (pg \cdot \mu g^{-1})\} \times \{[\text{phytoplankton biomass } (\mu g \cdot L^{-1})] \times 10^{-6}\ (g \cdot \mu g^{-1})\}. \quad (2)$$

Finally, the MCLR concentration is converted from $pg \cdot L^{-1}$ to $pg \cdot cell^{-1}$ using the equation,

$$\text{MCLR } (pg \cdot cell^{-1}) = [\text{MCLR } (pg \cdot L^{-1})] \div [\text{number of } \textit{Microcystis} \text{ cells} \cdot L^{-1}]. \quad (3)$$

A three-point running average was used to describe the temporal pattern of intracellular MCLR concentrations. Based on log-log transformed data, Pearson's correlation analysis ($P<0.05$; Wilkinson et al. 1992) was used to determine the significance of the relationships between: 1) phytoplankton volumes (particularly *M. aeruginosa*) and limnological parameters; and 2) volumes of different cyanobacterial genera and MCLR concentrations.

Results and Discussion

Succession of phytoplankton communities

Little Beaver, Coal, and Driedmeat lakes contained 26 genera of phytoplankton (Table 2), including representatives of Cyanophyceae (cyanobacteria), Chlorophyceae (chlorophytes), Cryptophyceae (cryptophytes), and Bacillariophyceae (diatoms). Chlorophytes represented the largest group of genera (46%) but contributed the least to the overall phytoplankton volume during most of the sampling periods. Phytoplankton volume was correlated with chl*a* concentrations over all three years and three study lakes (r=0.69, n=138, P<0.0001). Coincident with decreased total sunshine hours for the months of June to August in the three years (297, 267, and 263 h for 1991, 1992, and 1993, respectively; Environment Canada), there was a decrease in average June to August phytoplankton volumes and chl*a* concentrations (Fig. 2, 3) from 1991 to 1993 in Little Beaver and Coal lakes. In contrast, Driedmeat Lake chl*a* concentrations and phytoplankton volumes in 1992 and 1993 were higher than in 1991. In Driedmeat Lake, mean summer TP concentration increased from 208 $\mu g \cdot L^{-1}$ in 1991 to 503 $\mu g \cdot L^{-1}$ in 1993 (Fig. 4); nutrient loading from the nearby wastewater treatment plant may have had an impact on the intensity of phytoplankton blooms in Driedmeat Lake. Seasonal changes in chl*a* and TP concentrations were positively correlated within Driedmeat and Coal lakes (data pooled over the 3 yr: r=0.65 and 0.62; n=48 and 43, respectively; P<0.0001) and to a lesser extent positively correlated in Little Beaver Lake (r=0.38; n=41; P<0.02). The weaker correlation between TP and chl*a* in Little Beaver Lake may be related to the influence of moderate salinity in that lake (Table 1).

Succession of phytoplankton communities in these three eutrophic temperate lakes was atypical (Fig. 5). For example, in Driedmeat Lake, cryptophytes and diatoms were the dominant phytoplankton in early spring (May), cyanobacteria were the dominant phytoplankton in summer (June to August) and into the early fall (September), and diatoms returned in the late fall (October). Except on 25 July 1991 (72% of the phytoplankton volume), chlorophytes were relatively unimportant (a mean of ≤ 5% of the phytoplankton volume for May and June) in the phytoplankton community in Driedmeat Lake. In Little Beaver Lake, the maximum proportion of cyanobacteria decreased from 1991 to 1993 (from 100 to 67% of the phytoplankton volume, respectively) while chlorophytes increased from sporadically detectable in 1991 (≤ 5%) to as high as 77% of

the phytoplankton volume on 17 August 1993. In Coal Lake, both diatoms and crytophytes were the dominant species in spring and early June 1992-1993 (≥ 50% of the total phytoplankton volume; not sampled in June 1991); a small portion of chlorophytes (a mean of 10-12% of the phytoplankton volume for May and June) were also seen in 1992 and 1993. In Coal Lake, cyanobacteria dominated the 1991-1993 summer phytoplankton community, and together with diatoms, intermittently dominated the community in fall 1991-1992 (not sampled in September 1993). Thus, occurrence of cyanobacterial blooms in these lakes between May to October appears to be more predictable than blooms of chlorophytes, crytophytes and diatoms.

Cyanobacterial dominance in summer has commonly been attributed to high water temperatures (> 20°C; Okino 1973) and low TN:TP ratios (< 29; Smith 1983). Robarts and Zohary (1987) showed that cyanobacteria can maintain high growth rates at temperature where other phytoplankton begin to show temperature inhibition. Water temperature is also an indicator of several environmental factors such as the quality, intensity, and duration of irradiance, and the stability of the water column. In addition, cyanobacteria are capable of fixing N_2 which enables them to be superior competitors when TN:TP <29 (Smith 1983) or NO_3^-:TP <5 (McQueen and Lean 1987) which usually occur in summer. Cyanobacteria volume was positively and diatom volume was negatively correlated with water temperature (Table 3); diatom volume often peaks in early spring and late fall when lake mixing occurs. Water temperature was not related to volume of chlorophytes or cryptophytes (Table 3). TN:TP ratios were positively correlated with the volume of chlorophytes, but negatively correlated with the volume of cyanobacteria (r= 0.58 and -0.57, respectively; Table 3).

When data from all lakes were pooled, TN concentration was correlated with chlorophytes, but not the abundance of cyanobacteria, cryptophytes and diatoms (Table 3). In contrast, TP alone was strongly correlated with the abundance of cyanobacteria, and in order of decreasing r, chlorophytes, cryptophytes, and diatoms (Table 3). Although some cyanobacteria can fix N_2, a recent study in an oligotrophic lake showed that cyanobacteria (including *Aphanocapsa* sp. *Aphanothece* spp. and unknown colonial species) prefer NH_4^+ to N_2-fixation during late summer and fall (Parson and Parker 1993). The dominance of cyanobacteria in summer in the phytoplankton community in the three study lakes appears to be related to high TP concentrations, which influence TN:TP ratios (r=0.54 and -0.02, respectively). It has been shown for lakes in Alberta region that TP was a better predictor than TN:TP for cyanobacteria biomass prediction (Trimbee and Prepas 1987).

Succession of species within blooms of cyanobacteria

Despite the predictable summer blooms of cyanobacteria in these lakes, the succession of species within these blooms varied within a lake among years, and among lakes within a year. Over the 3-year study, the most important cyanobacterial species in terms of percent phytoplankton volume were *M. aeruginosa* and *Anabaena* spp. in Little Beaver Lake, *Aph. flos-aquae* and *Gomphosphaeria* sp. in Coal Lake, and *M. aeruginosa* and *Aph. flos-aquae* in Driedmeat Lake. Other cyanobacteria were noted only intermittently in these lakes.

In Little Beaver Lake (Fig. 6), the succession of cyanobacterial dominance in the summer blooms in 1991 was *Aph. flos-aquae* in June, *Anabaena* spp. in early July, and *M. aeruginosa* from mid-July to early fall (peaked at 90, 87, and 82% of phytoplankton volume, respectively). In 1992 however, *M. aeruginosa* was the single prominent species in summer (at times up to 89% of the phytoplankton volume) and persisted until early fall. *Aph. flos-aquae* appeared only intermittently (< 25% of the phytoplankton volume) in late July and early August 1992. In 1993, cyanobacteria decreased to <50% of the phytoplankton volume on most sampling dates. *M. aeruginosa* peaked in mid-June, *Anabaena* spp. dominated for most of the summer, and *Aph. flos-aquae* from late July and early August. Over the three years, the dominant species in Little Beaver Lake appeared to have shifted from *M. aeruginosa* (1991-1992) to *Anabaena* spp. (1993).

In Coal Lake (Fig. 7), *M. aeruginosa* peaked at early June and again in late July 1991 (55 and 52%, respectively), and was non-detectable from the lake in 1992 and 1993 (monthly mean < 2% of the phytoplankton volume). While *Gomphosphaeria* spp. and *Anabaena* spp. peaked sporadically in 1991-1992 and 1993, respectively, *Aph. flos-aquae* peaked at almost the same time each year. For example, *Aph. flos-aquae* peaked twice in summer 1991 (64% of the phytoplankton volume on 04 June and 82% on 08 August) and 1992 (47% on 02 July and 87% on 06 August). In 1993, *Aph. flos-aquae* decreased in abundance, and was replaced by *Anabaena* spp. from late June (49%) to early July (24%), and by *Lyngbya* sp. for the rest of the summer (ranged from 40 to 87%). Thus, *Aph. flos-aquae* was the dominant species in 1991 and 1993, and *Lyngbya* sp. was the dominant species in 1993.

In Driedmeat Lake (Fig. 8), the periodicity of cyanobacteria was again different. In 1991, *M. aeruginosa* was the only species to appear in June (up to 17% of the phytoplankton volume); it was replaced by *Anabaena* spp. in mid-July (87%), and *Aph. flos-aquae* thereafter (up to 89%). For the following two years, *Aph. flos-aquae* appeared in early June and dominated the blooms from July to early August. *M. aeruginosa* increased in abundance over the 3 years and became a co-dominant species with *Aph. flos-*

aquae in late August and early September (maximum of 58 and 56% for 1992 and 1993, respectively). In contrast, there was a decrease in the abundance of *Anabaena* spp. over the three years. From 1991-1993, the dominant species in Driedmeat Lake was *Aph. flos-aquae*; however, the sub-dominant species appeared to be less predictable and shifted from *Anabaena* spp. in 1991 to *M. aeruginosa* in 1992 and 1993. Thus, the periodicity of *Anabaena* spp. and *M. aeruginosa* in Driedmeat Lake were unpredictable.

Of all the chemical parameters tested (Table 4), the volume of *M. aeruginosa* in the study lakes was strongly positively correlated with TP concentration and pH, and to a lesser extent with CO_3^{2-}, NH_4^+, and TN concentrations, and was negatively correlated with TN:TP ratio and Secchi depth. Unlike previous reports, the volume of *M. aeruginosa* in these lakes was not correlated with water temperature (r= 0.04; n=138; P=0.63). The lack of correlation between water temperature and *M. aeruginosa* may in part due to its wide range of 'tolerance' growth temperatures (Fig. 9). In addition, the monthly mean water temperature in these lake never reached ≥ 25 °C, a temperature at which *M. aeruginosa* proliferated in Lake Kasumigaura (Komatsu 1980; Takahashi et al. 1981). Similar to a study of Saskatchewan lakes (Hammer 1969), water temperature in this study was positively correlated with *Aph. flos-aquae* volume and barely associated with *Anabaena* spp. volume (r=0.35; n=43; P=0.02 and r=0.23; n=43; P=0.14, respectively). The volume of *Aph. flos-aquae* peaked when water temperature was around 20°C whereas *Anabaena* spp. has similar phytoplankton volume between 12 and 20°C (Fig. 9). The volume of *Gomphosphaeria* sp. remained low in these lakes and peaked once at water temperature ≤ 10°C. Thus, *Aph. flos-aquae* appeared to proliferate at around 20°C whereas *M. aeruginosa* grew at a wide range of water temperatures.

As a result of tolerance to a wide range of water temperatures, the volume of *M. aeruginosa* was correlated with that of *Aph. flos-aquae* and *Phormidium mucicola* in the three study lakes (Table 5). Except on 07 August 1991 in Little Beaver Lake, *P. mucicola* contributed to < 7% of the phytoplankton volume in all the blooms in these 3 years. *M. aeruginosa* and *Anabaena* spp. volumes were not correlated. Interestingly, previous report suggested that the presence of *Microcystis* inhibits the growth of *Chlorella* sp. (chlorophyte) and *Anabaena* sp. The inhibitory effect of *Microcystis* is independent of nutrient concentration and may be due to the production of extracellular product(s) by *Microcystis* (Lam and Silvester 1979). This inhibitory extracellular product(s) has not yet been identified.

Cyanobacteria-MCLR relationship and its periodicity

MCLR concentrations in the three study lakes were positively correlated with *M. aeruginosa* and *P. mucicola* (Table 6). However, among all the cyanobacterial species present, only *M. aeruginosa* and *Ana. flos-aquae* have been reported to produce MCLR (Carmichael 1988). In addition, *P. mucicola* was not a dominant phytoplankton species in these lakes; the positive correlation between MCLR and *P. mucicola* might have been driven by the autocorrelation between *M. aeruginosa* and *P. mucicola* (Table 5). No significant relationship existed between MCLR and the relative proportion of *Anabaena* spp. and *Aph. flos-aquae* (r= -0.13 and 0.03, respectively), and MCLR was significantly negatively correlated with *Gomphosphaeria* sp. (Table 6). Thus, *M. aeruginosa* was probably the MCLR-producer in these lakes over the 3-year study.

The MCLR concentrations in these three lakes were variable within each lake and among years, reflecting the variability in both the abundance of *M. aeruginosa* and the production of MCLR by *Microcystis* cells within lakes and over time (Fig. 10). In Little Beaver Lake, MCLR increased from below detection in early June 1991 to 915 $\mu g \cdot g^{-1}$ biomass in early August 1991, remained high for the rest of the summer and into fall (711 to 923 $\mu g \cdot g^{-1}$), peaked on 09 October 1991 (1300 $\mu g \cdot g^{-1}$) and declined in the late fall. There was an overall decrease in mean summer MCLR concentrations for the 3 years (389, 251, and 32 $\mu g \cdot g^{-1}$ for 1991, 1992, and 1993, respectively), and MCLR and relative proportion of *M. aeruginosa* were only correlated in 1991 (r=0.82; n=13; P<0.001). This is partly because maximum MCLR concentration in Little Beaver Lake was not always found in summer when *M. aeruginosa* thrived. For example, MCLR concentration peaked in late fall in 1991 in Little Beaver Lake (1307 $\mu g \cdot g^{-1}$ on 09 October) when *M. aeruginosa* was only 51% of the phytoplankton volume; the maximum MCLR concentration in 1992 and 1993 was recorded in early spring (865 $\mu g \cdot g^{-1}$ on 12 May 1992 and 110 $\mu g \cdot g^{-1}$ on 25 May 1993) when *M. aeruginosa* was only 15 and 8% of the total phytoplankton volume, respectively. Thus, the decrease in MCLR over the 3 years in Little Beaver Lake was only partly explained by the variability in the relative proportion of *M. aeruginosa* (data pooled from all 3 years: r=0.56; n=45; P<0.0001).

The MCLR concentrations in Coal Lake were consistently lower than in both Little Beaver and Driedmeat lakes. In Coal Lake, MCLR concentrations were lowest in 1991 when only one sample was found to contain 1 $\mu g \cdot g^{-1}$, and ranged from 229 $\mu g \cdot g^{-1}$ and 50 $\mu g \cdot g^{-1}$ for 1992 and 1993, respectively. On dates when MCLR concentrations were low in 1992 and 1993, *M. aeruginosa* was absent from the lake; however, there was no correlation between MCLR and the relative proportion of *M. aeruginosa* from 1991-1993 in Coal Lake (data pooled from all 3 years: r=0.21; n=46; P=0.16). In Driedmeat Lake,

seasonal changes in MCLR were again different. In 1991, MCLR decreased from a maximum concentration of 722 $\mu g \cdot g^{-1}$ in early July to as low as 45 $\mu g \cdot g^{-1}$ in early August, and remained at 95 to 346 $\mu g \cdot g^{-1}$ thereafter. In 1992 however, MCLR concentrations in May and June were low (below detection to 14 $\mu g \cdot g^{-1}$), increased substantially in July and August (monthly mean of 101 and 662 $\mu g \cdot g^{-1}$), peaked on 25 August (938 $\mu g \cdot g^{-1}$), and gradually declined in late September. Similar changes in MCLR concentration were seen in 1993 except for higher monthly average MCLR concentrations (246, 706, and 567 $\mu g \cdot g^{-1}$ for July, August, and September, respectively). Changes in MCLR concentration in Driedmeat Lake were highly correlated with the relative proportion of *M. aeruginosa* in both 1992 and 1993 (pooled data from all 3 years: r=0.77; n=43; P<0.0001). When all data in these three lakes over the 3-year period were pooled, over 40% of the variability in MCLR could be explained by the abundance of *M. aeruginosa*.

When detectable MCLR ($\mu g \cdot g^{-1}$) was expressed as per 10^3 *Microcystis* cells, the periodicity of intracellular MCLR concentration in Little Beaver (1991-1993) and Driedmeat (1993) lakes can be categorized into three phases (Fig. 11). During phase 1, intracellular MCLR concentration declined; this suggested that the production of *Microcystis* cells was faster than the synthesis of MCLR by cells. During phase 2, intracellular MCLR concentration was stable, possibly the rate of production of *Microcystis* cells was the same as the rate of synthesis of MCLR by cells. During phase 3, intracellular MCLR concentration increased which implied that the production of *Microcystis* cells was slower than the synthesis of MCLR by cells. Although this hypothesis still remain to be tested, laboratory studies using a single strain of *M. aeruginosa* have shown that MCLR production is related to the different growth phases of cells. Toxicity increased during the exponential phase (Gorham 1964), reached a maxima during the late stages of exponential growth (Watanabe et al. 1989), and then dropped by more than 50% per day at the beginning of the stationary phase (Eloff and Van der Westhuizen 1980). In addition, Shirai et al. (1991) reported the ratio of toxic to non-toxic strains isolated from blooms varied from 6 to 86 percent; result based on 68 strain of *Microcystis* isolated from Lake Kasumigaura between 1986 and 1988. This may in part explain why some of the samples containing *Microcystis* did not have detectable MCLR concentrations. Furthermore, environmental parameters such as light (Watanabe and Oishi 1985; Van der Westhuizen and Eloff 1985a; Wicks and Thiel 1990), water pH (Van der Westhuizen and Eloff 1985b; Wicks and Thiel 1990), and nutrients (Codd and Poon 1988; Wicks and Thiel 1990) may have an effect on the short-term changes in toxicity observed in natural blooms. In the present three study lakes, MCLR concentrations were

positively correlated with TP, TDP, and chl*a* concentrations, and water pH (Kotak et al. submitted; Table 7).

In summary, seasonal changes in *M. aeruginosa* and MCLR concentrations were variable both among lakes and years; however, the notion that blooms may be toxic one day and not the next was not supported in our study. As the production of MCLR by *Microcystis* cells appears to vary over the lifetime of a bloom, direct monitoring of MCLR concentrations in drinking water lakes is needed for health risk assessments.

Table 1. Characteristics of the three study lakes, Little Beaver, Coal, and Driedmeat.

	Little Beaver	Coal	Driedmeat
Water supply for	Ferintosh	Wetaskiwin	Camrose
Population	200	10,000	15,000
Average depth (m)	~ 2	3.5	2.2
Surface Area (km^2)	1.53	10.9	16.5
Volume (m^3)	$\sim 3.1 \times 10^6$	38.8×10^6	41.9×10^6
Conductivity ($\mu S.cm^{-1}$)	1223	491	551

Table 2. The 26 genera of phytoplankton recorded in Little Beaver, Coal, and Driedmeat lakes from 1991-93.

Cyanobacteria	Chlorophytes	Cryptophytes	Diatoms
Anabaena	*Actinastrum*	*Cryptomonas*	*Asterionella*
Aphanizomenon	*Ankistrodesmus*	*Rhodomonas*	*Cyclotella*
Chroococcus	*Ankya*		*Stephanodiscus*
Gomphosphaeria	*Clamydomonas*		Unidentified
Lyngbya	*Coelastrum*		
Merismopodia	*Gemellicystis*		
Microcystis	*Oocystis*		
Phormidium	*Pediastrum*		
	Scenedesmus		
	Selenastrum		
	Tetraedron		
	Tetrastrum		

Table 3. Pearson correlation coefficients for phytoplankton cell volume ($\mu m^3 \cdot mL^{-1}$) versus water temperature (Water Temp.), total phosphorus (TP), total nitrogen (TN), TN:TP ratio, and nitrate+nitrite:TP (NO_3^-:TP) ratio for Little Beaver, Coal, and Driedmeat lakes from 1991-93. Correlation analysis was based on log-log transformed data.

Parameter		*r*	*n*	*P*
Water Temp. (°C)	cyanobacteria	0.38	138	<0.0001
	chlorophytes	-0.06	138	0.50
	cryptophytes	-0.002	138	0.98
	diatoms	-0.43	138	<0.0001
TP ($\mu g \cdot L^{-1}$)	cyanobacteria	0.54	139	< 0.001
	chlorophytes	-0.46	139	< 0.001
	cryptophytes	-0.19	139	< 0.03
	diatoms	0.21	139	< 0.02
TN ($\mu g \cdot L^{-1}$)	cyanobacteria	-0.02	39	0.92
	chlorophytes	0.35	39	< 0.03
	cryptophytes	-0.01	39	0.98
	diatoms	0.39	39	0.01
TN : TP	cyanobacteria	-0.57	39	<0.0002
	chlorophytes	0.58	39	<0.0001
	cryptophytes	0.18	39	0.29
	diatoms	-0.14	39	0.41
NO_3 : TP	cyanobacteria	-0.19	76	0.10
	chlorophytes	-0.10	76	0.37
	cryptophytes	-0.02	76	0.87
	diatoms	-0.10	76	0.40

Table 4. Pearson correlation coefficients for *Microcystis aeruginosa* cell volume ($\mu m^3 \cdot mL^{-1}$) versus environmental variables for Little Beaver, Coal, and Driedmeat lakes from 1991-93. Correlation analysis was based on log-log transformed data.

Parameter	*r*	*n*	*P*
NO_3^- ($\mu g \cdot L^{-1}$)	-0.08	78	0.51
NH_4^+ ($\mu g \cdot L^{-1}$)	-0.18	135	<0.03
TN ($\mu g \cdot L^{-1}$)	-0.32	41	<0.04
TP ($\mu g \cdot L^{-1}$)	0.54	137	<0.00001
TDP ($\mu g \cdot L^{-1}$)	0.43	137	<0.0001
TN:TP	-0.71	39	<0.00001
NO_3^-:TP	-0.14	75	0.24
CO_3^{2-} ($mg \cdot L^{-1}$)	0.32	98	<0.001
HCO_3^- ($mg \cdot L^{-1}$)	-0.13	118	0.16
pH	0.52	137	<0.00001
Water Temp. (°C)	0.04	138	0.63
Secchi depth (m)	-0.50	127	<0.0001

Table 5. Pearson correlation coefficients for *Microcystis aeruginosa* cell volume ($\mu m^3 \cdot mL^{-1}$) versus cell volumme of co-existing cyanobacterial species for Little Beaver, Coal, and Driedmeat lakes from 1991-93. Correlation analysis was based on log-log transformed data and n=135.

Parameter	r	P
Gomphosphaeria sp.	0.01	0.93
Anabaena spp.	-0.05	0.57
Aphanizomenon flos-aquae	0.40	<0.0001
Phormidium mucicola	0.60	<0.0001

Table 6. Pearson correlation coefficients for Microcystin-LR ($\mu g \cdot g^{-1}$ biomass) versus the relative proportion of five genera of cyanobacteria (%) for Little Beaver, Coal, and Driedmeat lakes from 1991-93. Correlation analysis was based on log-log transformed data and n=135.

Parameter	r	P
Microcystis aeruginosa	0.63	< 0.0001
Gomphosphaeria sp.	-0.21	< 0.02
Anabaena spp.	-0.13	0.13
Aphanizomenon flos-aquae	0.03	0.76
Phormidium mucicola	0.39	< 0.0001

Table 7. Pearson correlation coefficients for the intracellular of MCLR concentration per *Microcystis* cell versus environmental variables for Little Beaver, Coal, and Driedmeat lakes from 1991-93. Correlation analysis was based on log-log transformed data.

Parameter	*r*	*df*	*P*
NO_3^- ($\mu g \cdot L^{-1}$)	0.02	71	0.88
NH_4^+ ($\mu g \cdot L^{-1}$)	-0.03	116	0.74
TN ($\mu g \cdot L^{-1}$)	-0.20	39	0.25
TP ($\mu g \cdot L^{-1}$)	0.21	117	<0.02
TDP ($\mu g \cdot L^{-1}$)	0.11	117	0.23
TN:TP	-0.62	37	<0.0001
NO_3^-:TP	-0.16	67	0.20
CO_3^{2-} ($mg \cdot L^{-1}$)	0.17	90	0.11
HCO_3^- ($mg \cdot L^{-1}$)	0.09	105	0.38
pH	0.23	115	<0.01
Water Temp. (°C)	-0.04	118	0.67
Secchi depth (m)	-0.16	121	<0.01

Fig. 1. Structure of microcystin-LR.

Fig. 2. Seasonal changes of volume of phytoplankton communities in Little Beaver, Coal, and Driedmeat lakes from 1991-1993.

Fig. 3. Seasonal changes of chlorophyll *a* concentrations in Little Beaver, Coal, and Driedmeat lakes from 1991-1993.

Fig. 4. Seasonal changes of total phosphorus concentrations in Little Beaver, Coal, and Driedmeat lakes from 1991-1993.

Fig. 5. Seasonal changes of relative proportion of phytoplankton communities in Little, Coal, and Driedmeat lakes from 1991-1993.

Fig. 6. Seasonal changes of water temperature and percentage (by volume) of *Microcystis aeruginosa*, *Gomphosphaeria* sp., *Anabaena* spp., and *Aphanizomemon flos-aquae* in Little Beaver Lake from 1991-1993.

Fig. 7. Seasonal changes of water temperature and percentage (volume) of *Microcystis aeruginosa*, *Gomphosphaeria* sp., *Anabaena* spp., and *Aphanizomemon flos-aquae* in Coal Lake from 1991-1993.

Fig.8. Seasonal changes of water temperature and percentage (volume) of *Microcystis aeruginosa*, *Gomphosphaeria* sp., *Anabaena* spp., and *Aphanizomemon flos-aquae* in Driedmeat Lake from 1991-1993.

Fig. 9. Relationship between water temperature and the percentage (volume) of *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Anabaena* spp., and *Gomphosphaeria* sp. Data are monthly means pooled from Little Beaver (■), Coal (❍), and Driedmeat (×) lakes from 1991-1993.

Fig.10. Seasonal changes in relative proportion of *Microcystis aeruginosa* and microcystin-LR concentrations per gram biomass (based on dry weight) in Little Beaver, Driedmeat, and Coal lakes from 1991-1993.

Fig.11. Seasonal changes of intracellular concentrations of microcystin-LR per *Microcystis* cell in Little Beaver, Driedmeat and Coal lakes from 1991-1993.

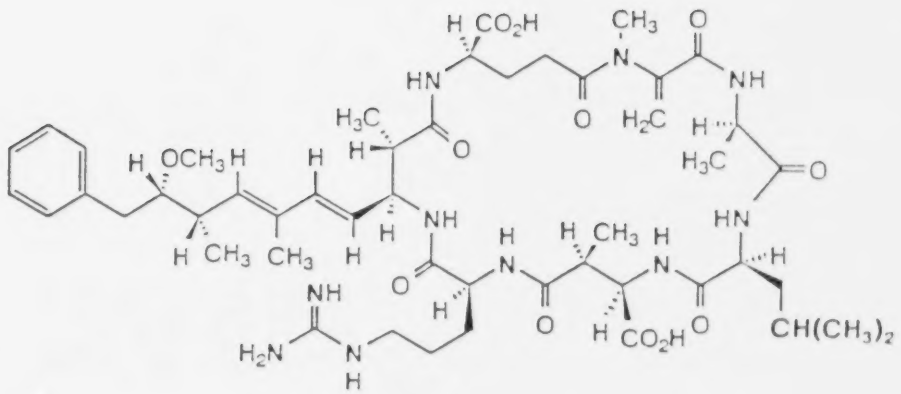

Fig. 1. Structure of microcystin-LR.

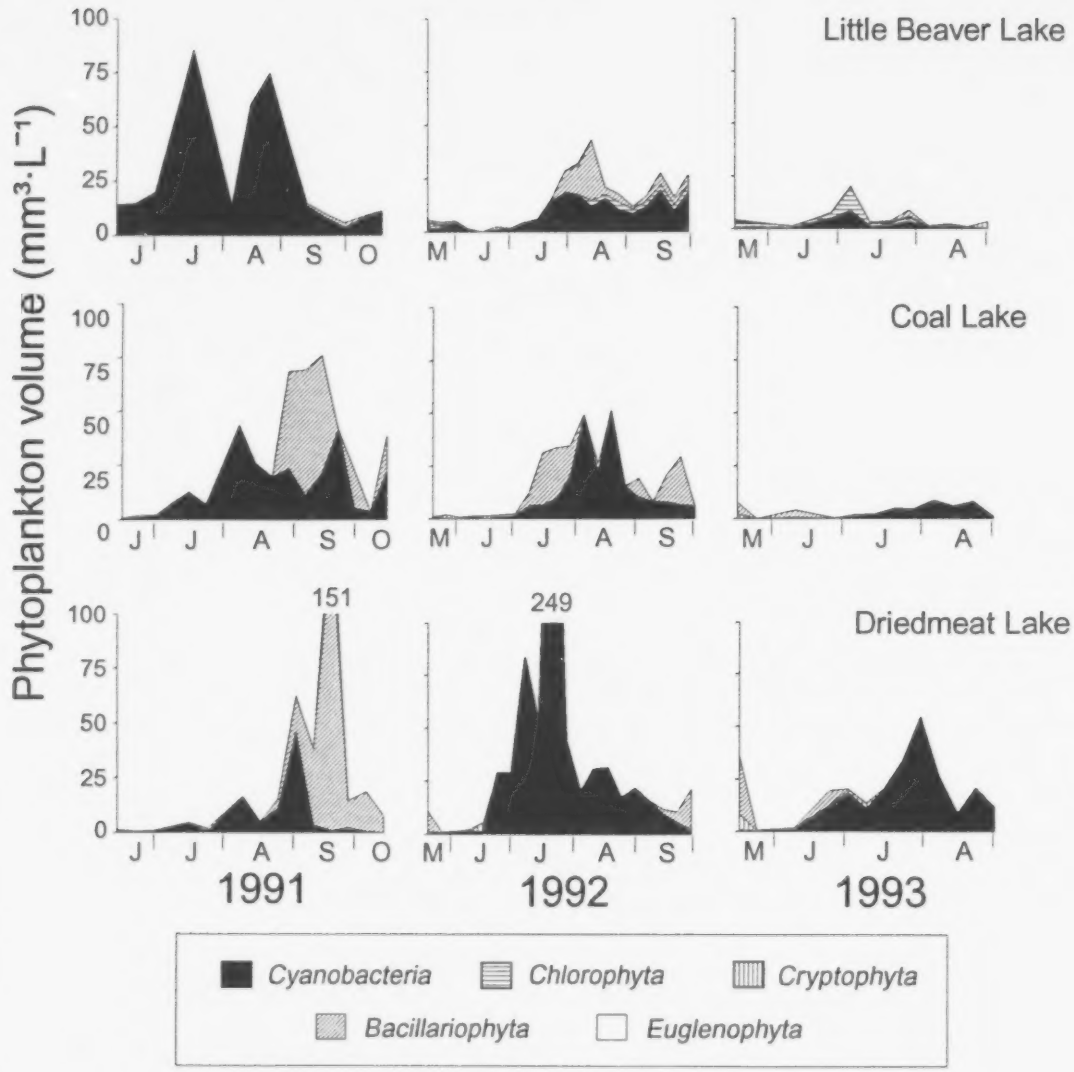

Fig. 2. Seasonal changes of volume of phytoplankton communities in Little Beaver, Coal, and Driedmeat lakes from 1991-1993.

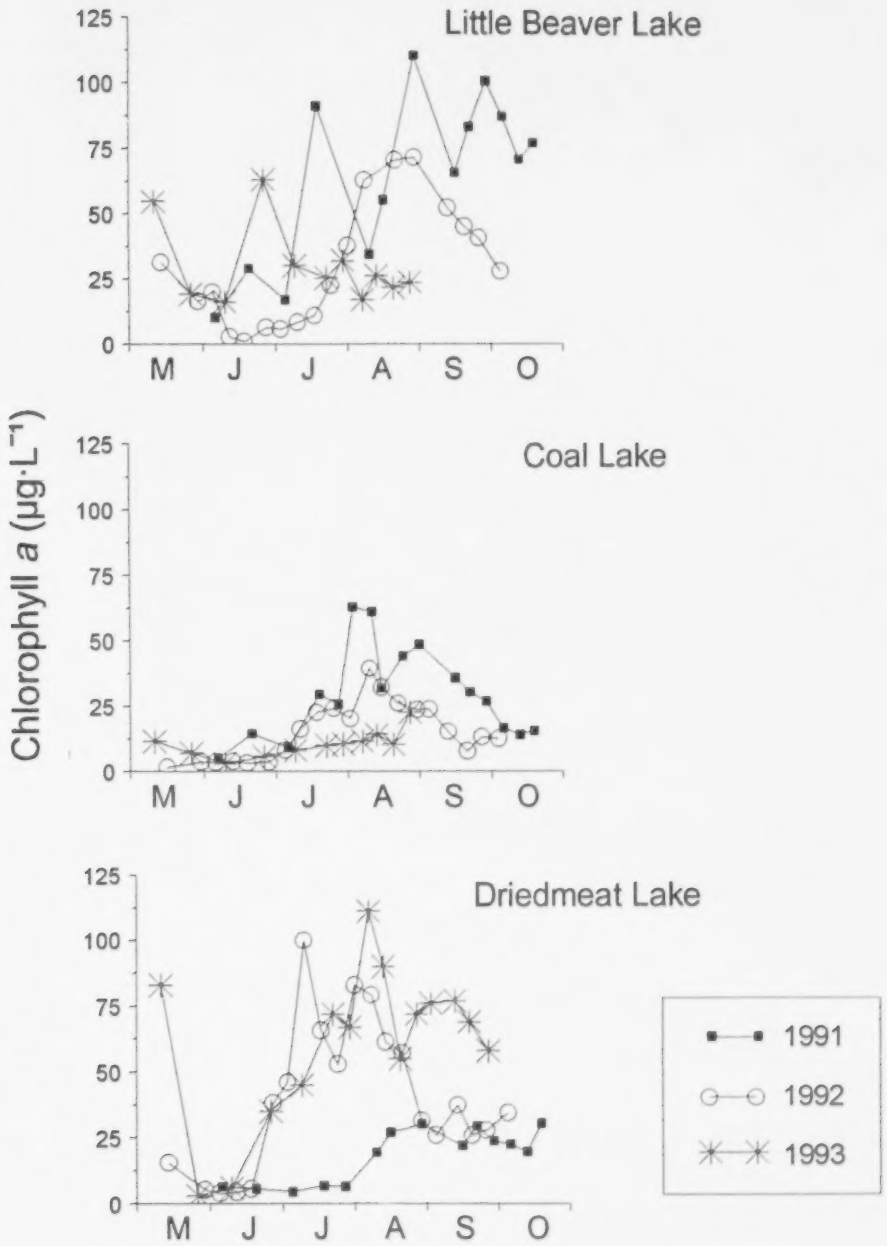

Fig. 3. Seasonal changes of chlorophyll *a* concentrations in Little Beaver, Coal, and Driedmeat lakes from 1991-1993.

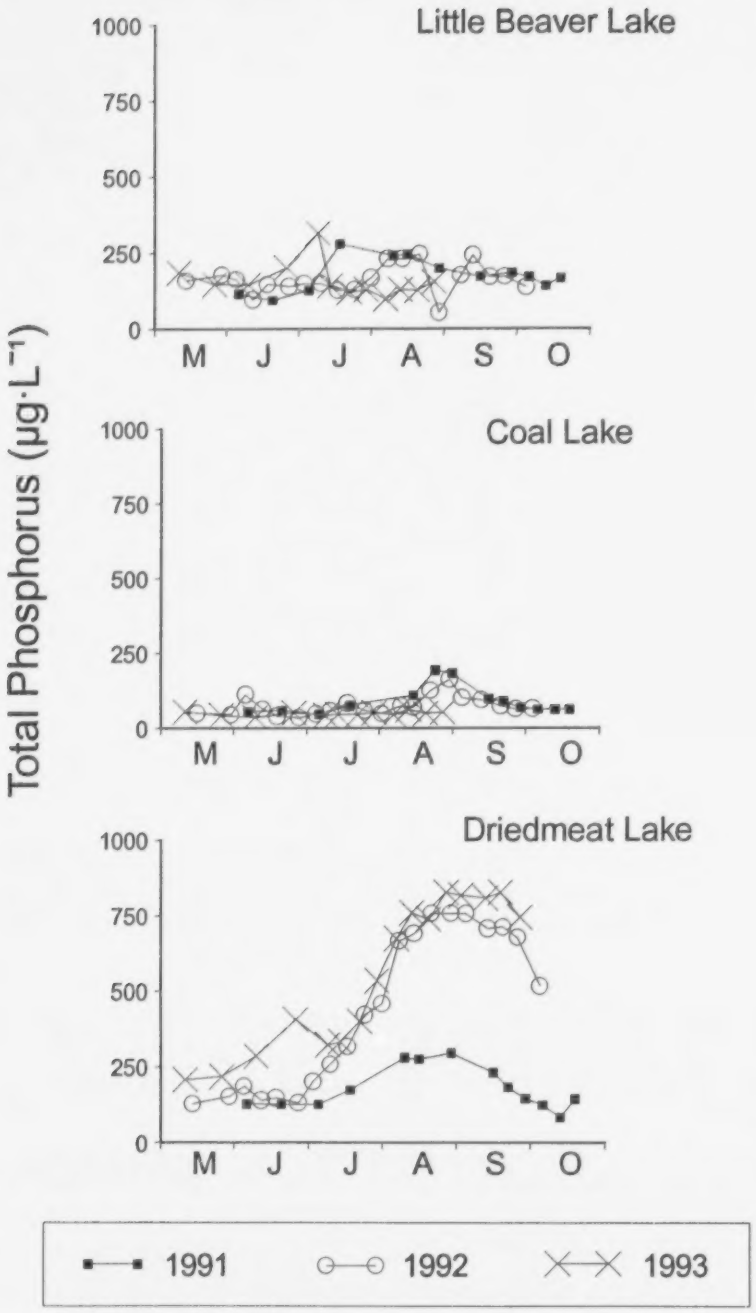

Fig. 4. Seasonal changes of total phosphorus concentrations in Little Beaver, Coal, and Driedmeat lakes from 1991-1993.

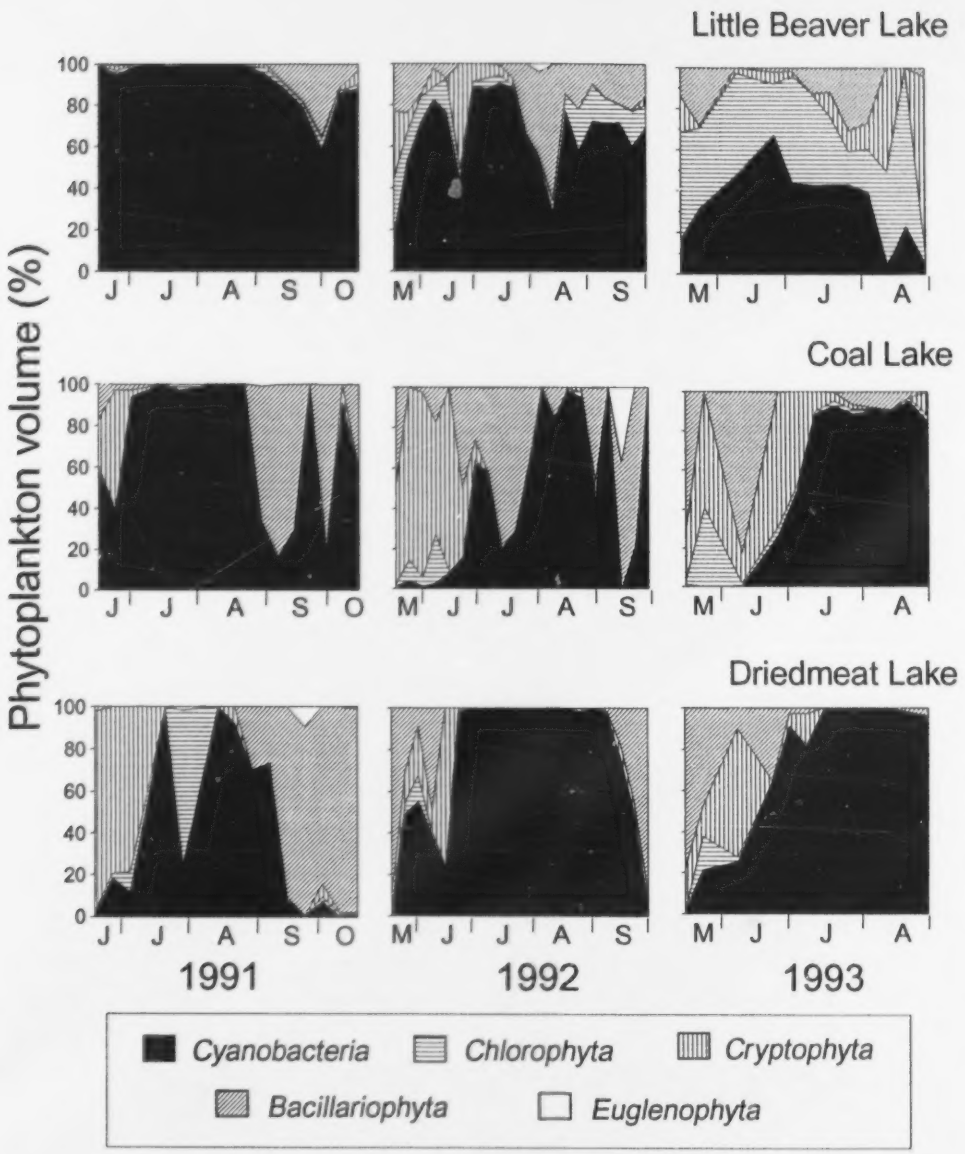

Fig. 5. Seasonal changes of relative proportion of phytoplankton communities in Little Beaver, Coal, and Driedmeat lakes from 1991-1993.

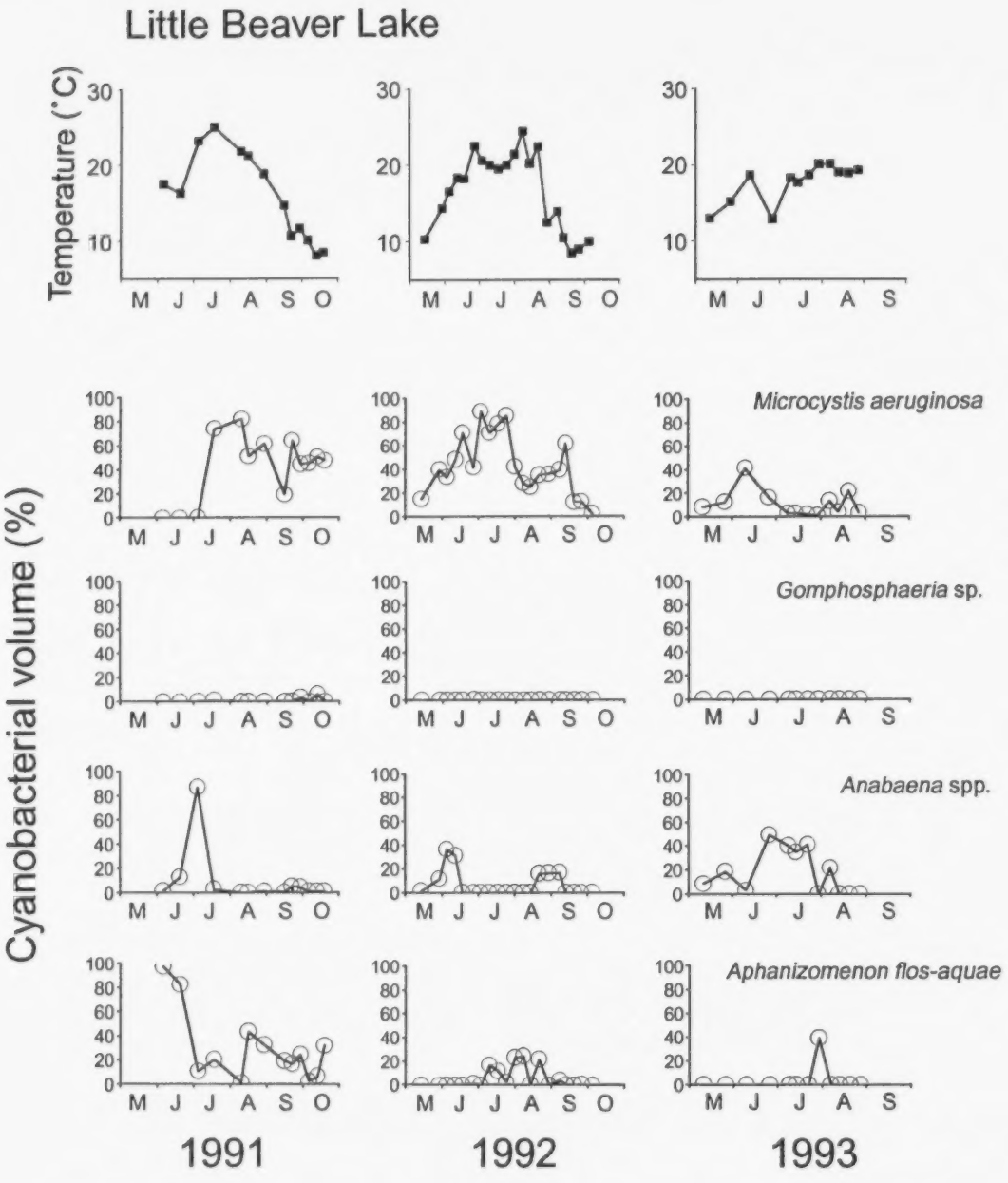

Fig. 6. Seasonal changes of water temperature and percentage (by volume) of *Microcystis aeruginosa*, *Gomphosphaeria* sp., *Anabaena* spp., and *Aphanizomemon flos-aquae* in Little Beaver Lake from 1991-1993.

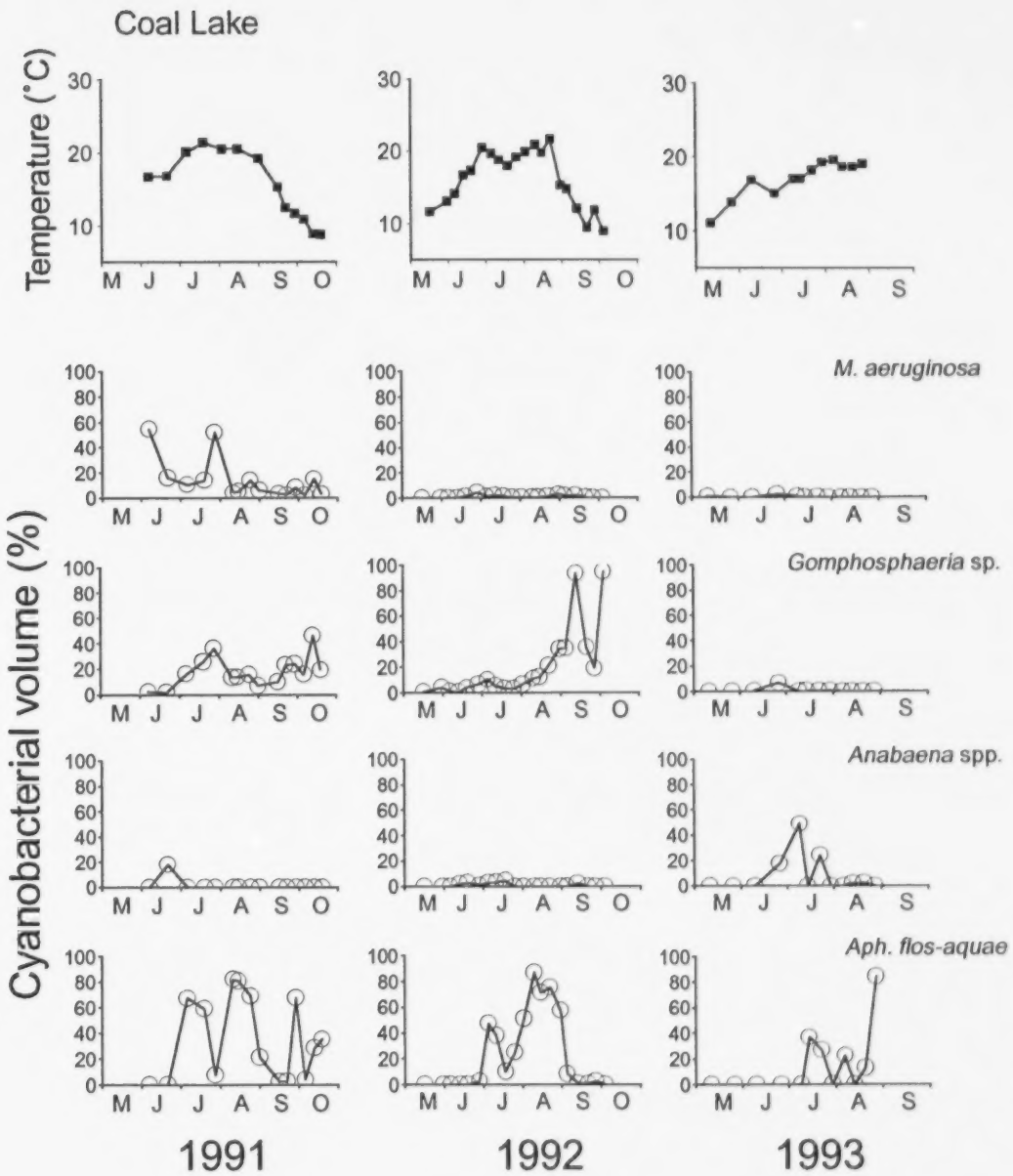

Fig. 7. Seasonal changes of water temperature and percentage (by volume) of *M. aeruginosa*, *Gomphosphaeria* sp., *Anabaena* spp., and *Aph. flos-aquae* in Coal Lake from 1991-1993.

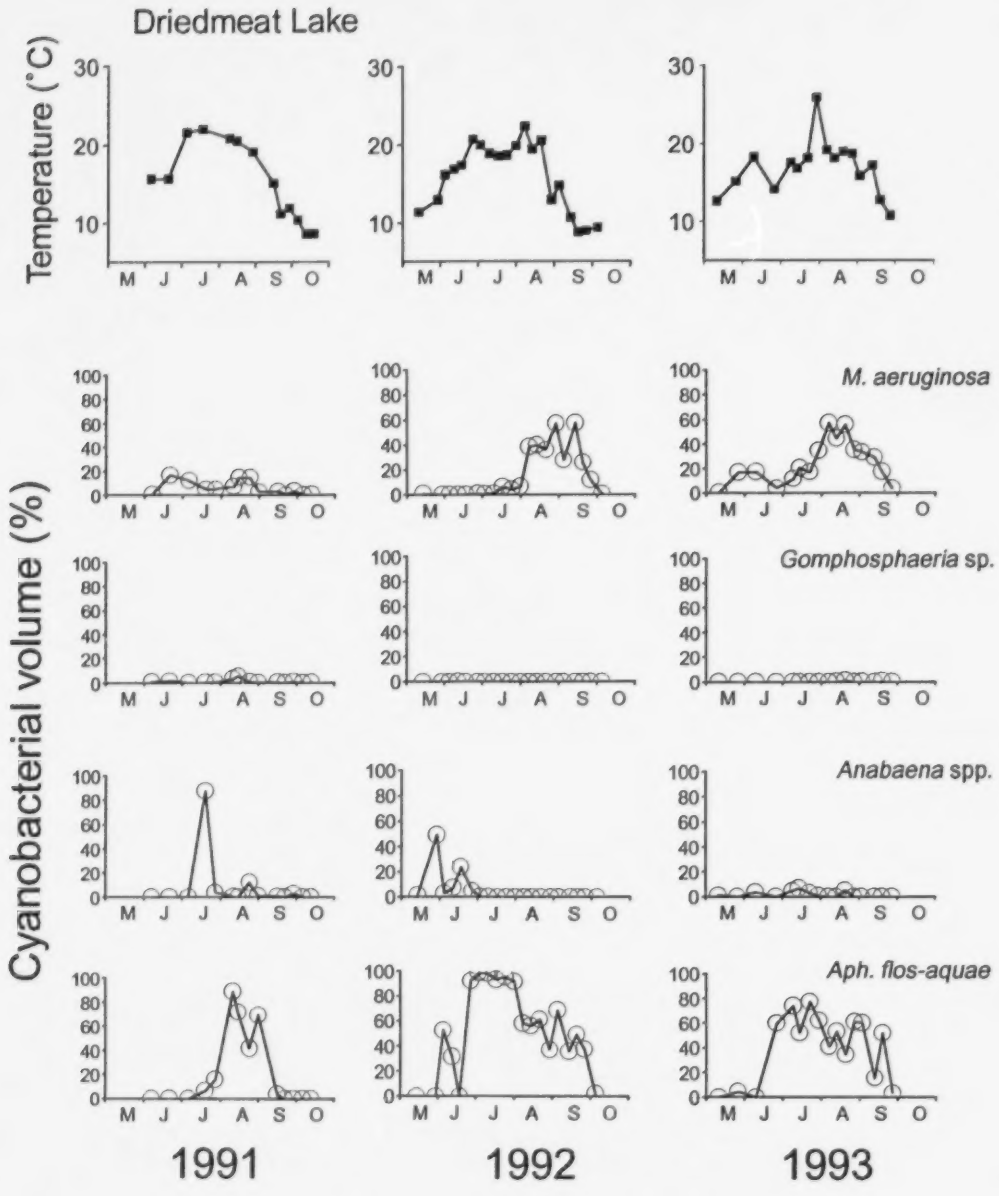

Fig. 8. Seasonal changes of water temperature and percentage (by volume) of *M. aeruginosa*, *Gomphosphaeria* sp., *Anabaena* spp., and *Aph. flos-aquae* in Driedmeat Lake from 1991-1993.

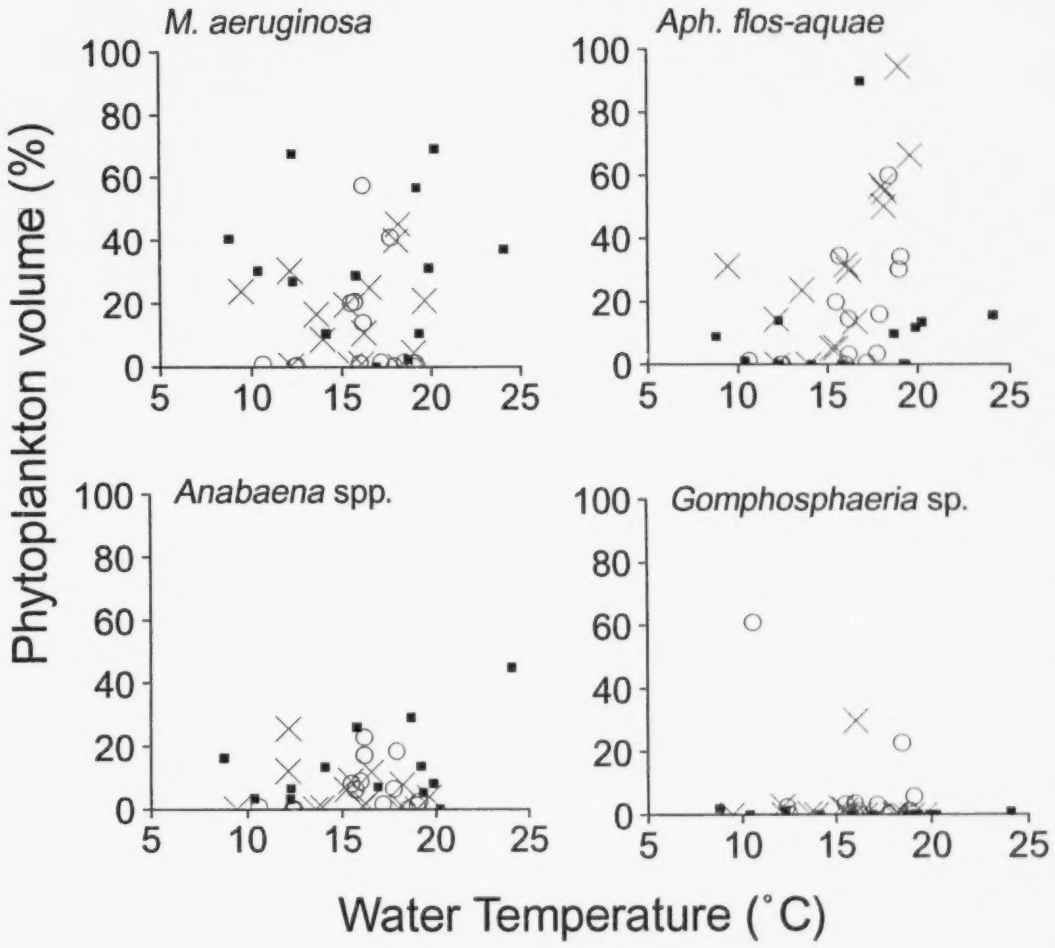

Fig. 9. Relationship between water temperature and the percentage (by volume) of *M. aeruginosa*, *Aph. flos-aquae*, *Anabaena* spp., and *Gomphosphaeria* sp. Data are monthly means pooled from Little Beaver (■), Coal (○), and Driedmeat (×) lakes from 1991-1993.

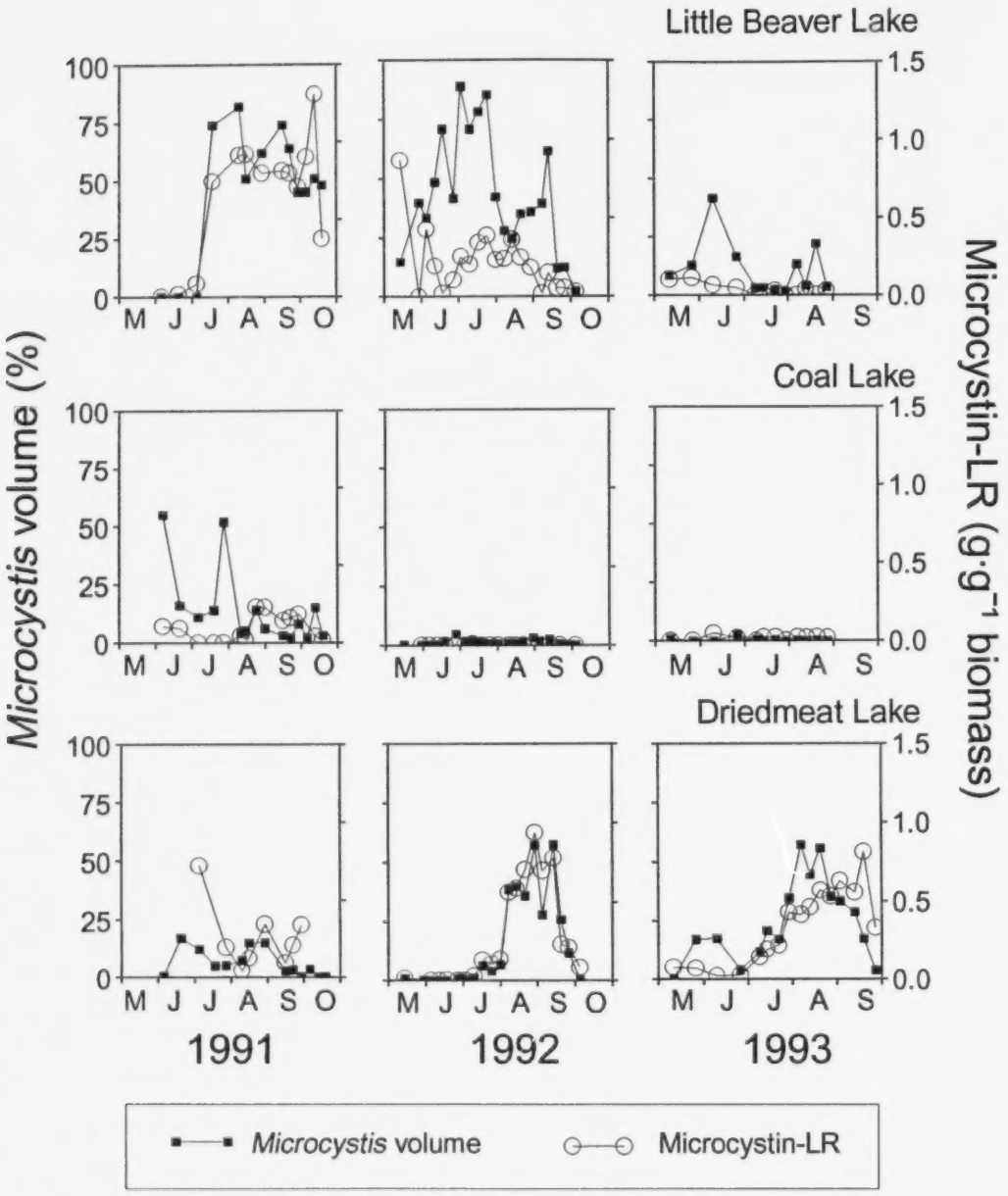

Fig. 10. Seasonal changes in relative proportion of *M. aeruginosa* and microcystin-LR concentrations per gram biomass (based on dry weight) in Little Beaver, Driedmeat, and Coal lakes from 1991-1993.

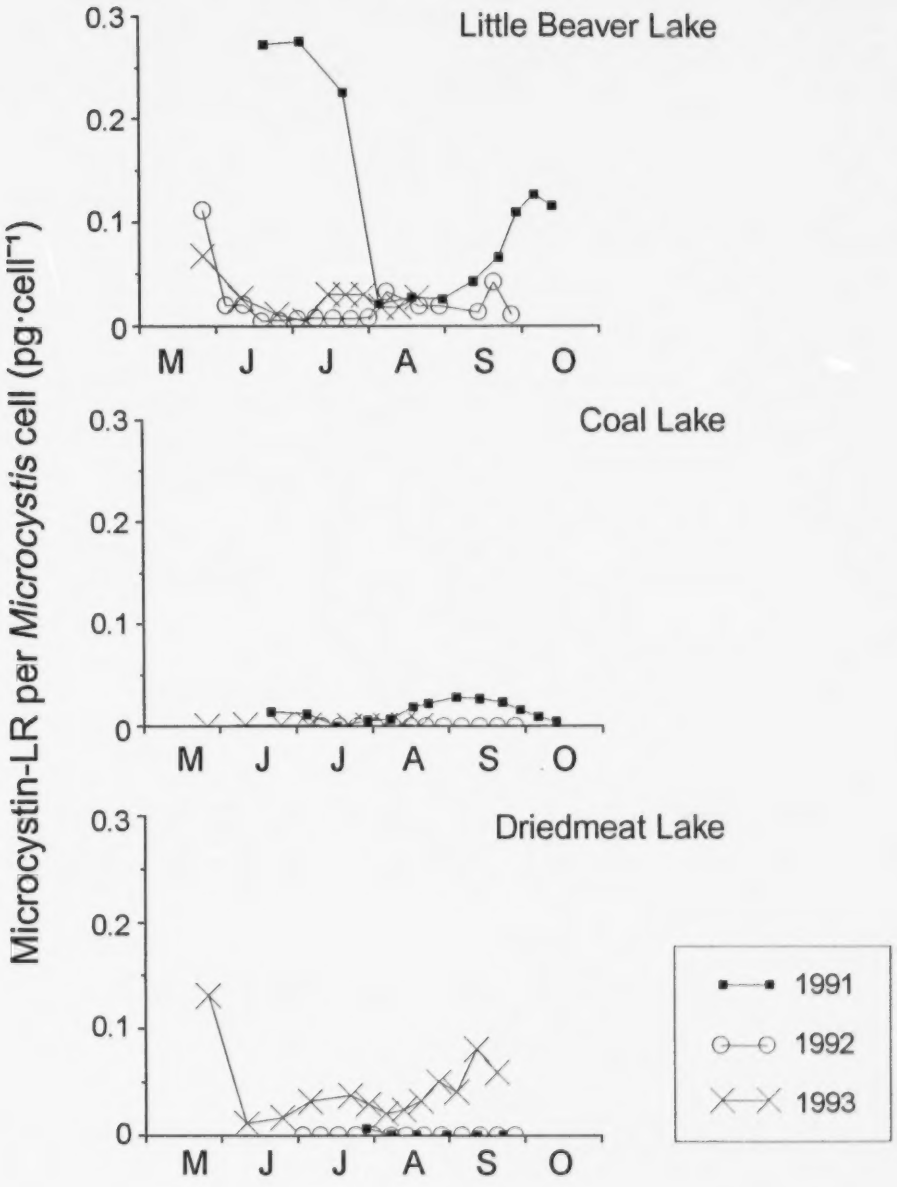

Fig. 11. Seasonal changes of intracellular concentrations of microcystin-LR per *Microcystis* cell in Little Beaver, Driedmeat and Coal lakes from 1991-1993.

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III. Chemical control of microcystin-containing phytoplankton blooms: Implications for human health[3]

Introduction

Cyanobacterial blooms and intracellular toxins

Eutrophication is common in lakes throughout the world as a consequence of low binding capacity of the bottom sediments for phosphorus (P) and/or high external inputs of nutrients (particularly P) (Schindler 1984). Phytoplankton (often cyanobacteria) thrive in eutrophic waters and decrease the economic value of recreational lakes. In central Alberta, half the lakes are eutrophic to hypereutrophic and blooms of cyanobacteria are common (Mitchell and Prepas 1990). In some cases, these lakes are the sources of drinking water for nearby communities.

Three genera of cyanobacteria, *Aphanizomenon*, *Anabaena*, and *Microcystis*, tend to dominate in phytoplankton blooms in central Alberta. These genera produce two types of intracellular toxins: alkaloid neurotoxins (*Anabaena* spp. and *Aphanizomenon* spp.) and cyclic hepatotoxins (*Microcystis* spp. and *Anabaena* spp.; Carmichael et al. 1985). In fresh water, anatoxin-*a* is the most commonly reported neurotoxin and microcystin-LR (MCLR) is the most commonly reported hepatotoxin. In this chapter, I focus on MCLR because of its potential to cause chronic human health problems and its prevalence in regional drinking water supplies (Kenefick et al. 1992; Kotak et al. 1993). The concentration of MCLR within phytoplankton cells and that released by phytoplankton cells into the water phase (dissolved) are referred to as endo-MCLR and exo-MCLR, respectively.

MCLR is a cyclic heptapeptide with a molecular weight of 995 daltons (Botes et al., 1985). MCLR is 86 times more toxic than sodium cyanide (Sax and Lewis 1989) and has

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3. A version of this chapter has been accepted for publication. A. K.-Y. Lam, E. E. Prepas, D. Spink, and S. E. Hrudey. *Water Res.*

been reported to be a potent liver tumor promotor (Nishiwaki-Matsushima et al. 1992). MCLR acts by specifically inhibiting the catalytic subunits of protein phosphatase 1 and 2A (PP-1c and PP-2Ac, respectively) (Fujiki 1992). Ingestion of an acute lethal dosage of MCLR by mice caused extensive liver haemorrhage (Konst et al. 1965; Kotak et al. 1993) and death within 1 to 3 h (Runnegar and Falconer 1981). Sublethal dosages of MCLR to mice elevated levels of various liver enzymes and caused cumulative liver damage (Falconer et al. 1988). Wildlife and domestic animal poisonings have occurred worldwide from the ingestion of MCLR-containing cyanobacteria (Galey et al. 1987; Ekman-Ekebom et al. 1992). Similarly, humans have become ill after swimming in water containing *Microcystis* spp. (Lawton and Codd 1991).

Cyanobacterial-dominated phytoplankton blooms tend to recur in a given water body. Although humans do not directly consume cyanobacteria, they may be regularly exposed to sub-lethal dosages of exo-MCLR in drinking waters derived from contaminated lakes and reservoirs. Thus, the release of MCLR by cyanobacteria in drinking water reservoirs has raised health concerns (Falconer et al. 1983).

Chemical control of phytoplankton blooms

Chemicals are used to control phytoplankton-related problems in surface waters and water treatment plants. Copper sulfate ($CuSO_4$) and Reglone A (diquat, 1,1-ethylene-2,2-dipyridilium dibromide) are frequently used for phytoplankton control in lakes and farm drinking water dugouts. The latter is thought to degrade rapidly (Alberta Agriculture 1989). Simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) is also used for phytoplankton control, particularly in recreational swimming ponds and farm dugouts (Walker 1964; Blackburn and Taylor 1976). Potassium permanganate ($KMnO_4$) is applied to control odour and other phytoplankton-related problems in muncipal drinking water supplies (Cherry 1962; Fitzgerald 1966; Montgomery 1985). Chlorination (NaOCl) is a disinfectant in water treatment plants to reduce biological activity. All of these chemicals remove phytoplankton by rupturing cells through either inhibition of new cell wall synthesis, enzymatic reactions, or photosynthesis.

However, alum ($Al_2(SO_4)_3 \cdot 14H_2O$) (Morency and Edwards 1985) and lime ($Ca(OH)_2$) (Murphy et al. 1990; Prepas et al. 1990) have been added to remove P in lakes and dugouts, since phytoplankton biomass is usually proportional to total phosphorus concentration (TP) (Schindler 1984). In water treatment plants, alum is often used as a coagulant and lime as a softener. Recommended dosages of treatment chemicals are based primarily on: 1) growth response of phytoplankton to the chemicals (Paterson and Wright 1988), 2) water chemistry (Kennedy and Cooke 1982), and/or 3) toxicity of the residual chemical (Worthing and Hance 1991). Treatment with copper sulfate causes lysis of *Microcystis* cells and subsequent release of MCLR (Jones and Orr 1994, Kenefick et al. 1993), whereas lime treatment results in no increase in exo-MCLR concentration in water (Kenefick et al. 1993). The reported impacts of copper sulfate treatment are epidemiologically related to the outbreak of human hepatoenteritis in Australia in 1979, after the application of copper sulfate to a toxic cyanobacterial bloom in a drinking water supply (Falconer et al. 1983). The effect of other commonly-used chemicals on the amount and rate of toxin release from *Microcystis* and other cyanobacteria has not been reported.

Purpose of study

The purpose of this study was to determine the effect of commonly-used treatment chemicals on the release of MCLR from cyanobacterial cells into the water. In particular, to find out whether or not chemicals which control cyanobacterial blooms through inhibition of new cell wall synthesis, enzymatic reactions, or photosynthesis (*e.g.*, Reglone A, $KMnO_4$, NaOCl, and Simazine) cause cell lysis and should increase exo-MCLR concentration in the surrounding water. In contrast, chemicals which control cyanobacterial blooms through nutrient precipitation and cell coagulation (*e.g.*, alum and lime) should not cause a significant increase in exo-MCLR concentration in the water column. I also determined 1) the half-life of exo-MCLR in lake water, 2) the persistence of endo-MCLR in decaying phytoplankton, and 3) the predictability of exo-MCLR concentrations based on nutrient concentrations in lake water.

Materials and Methods

Microcystin-LR in the water phase

Phytoplankton bloom material (primarily *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*) was collected from two lakes which serve as domestic water supplies. Little Beaver Lake is the water supply for the Town of Ferintosh and was sampled on 19 August 1991 (Batch 1); Driedmeat Lake is the water supply for the City of Camrose and was sampled on 19 August 1992 and 03 September 1992 (Batches 2 and 3, respectively). Driedmeat and Little Beaver lakes are situated in the Battle River drainage basin in central Alberta. Both are hardwater lakes; total alkalinity in the eutrophic zone averages 233 and 329 meq.$\cdot L^{-1}$ $CaCO_3$, respectively, and pH is often above 9 in the surface water in summer. These lakes have been monitored for toxic phytoplankton blooms since 1990. For this experiments, phytoplankton were collected with a conical 64-µm mesh net (30 cm diameter, 100 cm length) fitted with a brass bucket. Concentrated phytoplankton were re-suspended in 20-L Nalgene jars with lake water and brought to the laboratory the same day.

The experiments were performed in controlled environmental chambers at 24 $\pm$ 2 °C. Light intensity was adjusted to 32 µE$\cdot cm^{-2}sec^{-1}$ in a 16:8 h light:dark cycle. The phytoplankton mixture was evenly distributed among borosilicate glass jars. Two treatments for Batch 1 were in 25-L glass jars (one lime treatment and one 25-L control), the remainder of treatments (including a second control) for Batch 1 and all treatments for Batches 2 and 3 were in 15-L glass jars. All jars were placed on shakers for the duration of the experiment.

Before chemical addition, phytoplankton biomass was estimated in each jar. A sample of lake water was taken from each jar, freeze-dried and then weighed (about 1g$\cdot L^{-1}$ phytoplankton dry weight). To provide a detectable supply of exo-MCLR over the duration of the experiment, phytoplankton biomass in the jars was about 80-fold higher than that found in the lake. The dosage of chemicals used in this experiment was based on the removal of phytoplankton and consequently was higher than the recommended values (Table 1). Reglone A was added twice for Batch 3: once on day 0 and again on day 3 to

remove all phytoplankton. Single chemical dosages were administered to all other jars. To mimic the floating mat of cyanobacterial-dominated phytoplankton, each jar was placed in the dark for 12-15 h to allow the cyanobacteria to float to the surface of the water. After the phytoplankton mat formed, one of the six chemicals (Reglone A, NaOCl, $KMnO_4$, Simazine, alum, and lime) was added to each experimental jar; no chemical addition was applied to the control. All treatments in Batches 2 and 3 had two replicates, no treatment was replicated in Batch 1.

Composite water samples were collected from each jar with a peristaltic pump for both toxin and other nutrient analyses. For MCLR analysis, 400 to 650 mL of water was filtered through glass fibre filters (Whatman GF/C) and the filtrate was concentrated with a disposable 1-g C18 Sep-Pak cartridge (Supelco) prior to analysis by reversed-phase high performance liquid chromatography (HPLC; Kenefick et al. 1993). In this chapter, the detection limit for MCLR was 0.25 $\mu g \cdot L^{-1}$. Samples were collected immediately before and after chemical treatment, daily from day 1 to 3, at two- to three-day intervals from day 4 to 14, and at five- to seven-day intervals thereafter until exo-MCLR became non-detectable. The alum- and lime-treated, and control jars were monitored for 49 d (Batch 1), and 26 d (Batch 3). The remaining jars in all Batches were monitored for 14 d following treatment. In all jars, pH was measured to ensure that the treated water was within pH 6-10. The water was also analyzed for five nutrient fractions: total dissolved phosphorus concentrations (TDP; Prepas and Rigler 1982) in all jars, nitrate+nitrite concentrations (NO_3^-; Stainton et al. 1977) only in Batches 1 and 2, TP (<110 µm; Prepas and Rigler 1982), ammonium (NH_4^+; Solorzano 1969), and dissolved organic carbon concentrations (DOC; Ionics Corporation 1555 DOC analyzer with 100% Pt catalyst) in Batches 2 and 3.

Microcystin-LR inside cyanobacterial cells

To evaluate whether toxin release was the result of cell rupture, the surface layer of the phytoplankton bloom material before and after chemical treatment in Batch 1 was analyzed for the presence of MCLR. Approximately 0.07 to 0.10 g of freeze-dried phytoplankton was extracted with 5% acetic acid for toxin analysis as described by

Kenefick et al. (1992). The presence of MCLR as indicated by HPLC analysis, was consistent with the results of mouse bioassay and confirmed with fast atom bombardment mass spectrometry analysis of phytoplankton collected from the same lakes (Kotak et al. 1993). As there was a higher relative proportion of *Aph. flos-aquae* in Batches 2 and 3 than in Batch 1, all the surface phytoplankton cells in Batches 2 and 3 sank to the bottom of the jars after chemical treatments. To assess the persistence of toxin in decaying phytoplankton, the bottom 'phytoplankton scum' layer in Batch 2 (Reglone A, NaOCl, alum, lime and control) and Batch 3 (Reglone A, alum, lime and control) was collected for toxin analyses. At the end of the experimental period, the endo-MCLR concentrations in the floating phytoplankton in Batch 1 and the settled cyanobacteria in Batches 2 and 3 were also measured.

The amount of endo- and exo-MCLR after chemical treatment was expressed as a percent of detectable endo-MCLR at day 0 (*i.e.*, before chemical treatment) to allow comparison among batch experiments:

$$\text{MCLR released at day X (mg)} = \frac{\text{Exo-MCLR at day X (mg)}}{\text{Endo-MCLR at day 0 (mg)}} \times 100\%$$

With the exception of Batch 1 (n=1), *SE* for the endo- and exo-MCLR were calculated. These percentages could be applied as predictive tools to determine exo-MCLR concentrations when endo-MCLR concentrations are known.

Integrity of Microcystis *cells after chemical treatments*

A strain of *M. aeruginosa* was isolated from Pretzlaff dugout (Murphy et al. 1990), for confirmation as a MCLR-producer and for the study of cell integrity after chemical treatment. The culture was grown in Chu-10 medium (Chu 1942). When the culture reached its stationary phase, a chemical such as Reglone A, NaOCl, alum, or lime was added. After 24 h, each of the chemically-treated cultures was centrifuged, pre-fixed with buffered 2.5% glutaraldehyde (pH 7.2) and post-fixed with buffered 1% Osmium tetraoxide at room temperature. The pellets were then divided into two portions. One portion of the culture pellets was partially blotted with GF/C filter papers, dehydrated

through a series of 50 to 100% ethanol solutions, and critical-point dried at 41°C. The filter papers were then mounted on stubs, coated with gold (Edward S150B Sputter Coater), and examined in a scanning electron microscope (SEM; Hitachi S-2500). The other portion of the culture pellets were dehydrated in a series of ethanol solutions, and embedded in Araldite CY212 mixture overnight in a vacuum desiccator. The mixture was subsequently polymerized at 60°C for 2 d, thin-sectioned to 1 μm, and examined in a transmission electron microscope (TEM; Hitachi H-7000).

Results and Discussion

Control jars

All three batch experiments contained approximately the same amount of phytoplankton bloom material, and were co-dominated by *M. aeruginosa* and *Aph. flos-aquae*. The initial endo-MCLR concentration in Batch 1 was 3 to 4-fold higher than in Batches 2 and 3, respectively, at least partially because the proportion of *M. aeruginosa* at day 0 was higher in Batch 1 than in Batches 2 and 3 (Table 2). In contrast, the initial exo-MCLR concentrations were similarly low or undetectable in all three Batches (Fig. 1). Thus, almost all MCLR was inside the phytoplankton cells at the beginning of the experiments.

Average exo-MCLR maximum in all control jars (9.9 ± 0.8%) were lower than in all chemically-treated jars in most of the experimental periods (Fig. 1). Although the exo-MCLR in Batches 1 and 3 was monitored for a longer time (up to 49 and 26 d, respectively) than for Batch 2 (12 d), there was no noticeable change in the exo-MCLR concentration after day 12 in Batches 1 and 3. The maximum percentage of exo-MCLR in all control jars ranged from 9.4 ± 4.0% in Batch 3 to 14.3 ± 0.1% in Batch 1. The percent by volume of *M. aeruginosa* in both the 15- and 25-L glass jars of Batch 1 was similar at the beginning (98 and 97%, respectively) and at the end (100% for both) of the experimental period. Thus, the release of MCLR from phytoplankton into the surrounding water was probably due to changes in cell permeability resulting from cell aging (Berg et al. 1987) or bacterial-lysis (Shilo 1971; Steward and Daft 1977).

By the end of the experimental period, the endo-MCLR concentrations in the floating phytoplankton (Batch 1) and in the decaying phytoplankton (Batches 2 and 3) were different (Fig. 2). For the floating phytoplankton, there was a 2.3- and 1.7-fold increase in endo-MCLR per gram of bloom material (by dry weight) in the 15- and 25-L control jars, respectively. As there was no substantial change in the proportion of *Microcystis* by cell volume over the experimental period, the increase in endo-MCLR concentrations in Batch 1 suggests that the cells were producing MCLR. The DOC and NO_3^- (data not shown) concentrations, and pH of lake water in the control jars were stable. The NH_4^+, TDP and TP concentrations in the control jars, however, increased over the experimental period (Fig. 3). Thus, the production of MCLR by *M. aeruginosa* was not likely in response to a lack of N or P. These results are consistent with the nutrient-deficiency laboratory experiments by Watanabe and Oishi (1985): a decrease in N and P concentrations in culture medium reduced *M. aeruginosa* growth significantly but had comparatively little effect on *M. aeruginosa* toxicity based on a mouse bioassay.

There was an overall decrease in endo-MCLR concentrations as the phytoplankton decayed. The fractions of the initial endo-MCLR remaining in the decaying phytoplankton of Batches 2 and 3 after 2 weeks were 39 ± 4 and 80 ± 13%, respectively. Thus in a lake setting, endo-MCLR may remain at detectable concentrations for a substantial period and degrade gradually within phytoplankton cells.

Treatment with Reglone A, NaOCl, $KMnO_4$, or Simazine

The patterns of endo- and exo-MCLR concentrations in all jars treated with Reglone A, NaOCl, $KMnO_4$, or Simazine were similar. Before chemical addition, MCLR was present in the floating phytoplankton mat but was undetectable or at very low concentrations in the water phase (Fig. 1). After chemical addition, endo-MCLR concentrations in both the floating and decaying phytoplankton were significantly lower than in the control jars (Fig. 2). In the water phase, the toxin was detected in varying concentrations; generally, exo-MCLR reached a maximum between days 1 and 3, and then declined over time (Fig. 1).

Addition of Reglone A or NaOCl to concentrated phytoplankton blooms containing primarily cyanobacteria resulted in more detectable exo-MCLR in the water phase than in the control jars (Fig. 1). This result suggests that both Reglone A and NaOCl may induce release of MCLR from phytoplankton more than the 5% acetic acid used in the present endo-MCLR extraction method. The extraction method used in this study is very reproducible (*C.V.* = 3.7%, n=6; Hrudey et al. in press). Thus, the incomplete extraction of MCLR from particulate matter does not impact on the conclusion of this comparative study.

Exo-MCLR maxima varied among Batches (Fig. 1). A higher endo-MCLR concentration (or proportion of *Microcystis* cells) on day 0 generally resulted in an overall higher exo-MCLR concentration for the same chemical treatment. For example, in the jar treated with Reglone A, the exo-MCLR maximum measured in Batch 1 (2.01 $mg \cdot L^{-1}$) was higher than in Batches 2 (0.26 ± 0.03 $mg \cdot L^{-1}$) and 3 (0.13 ± 0.02 $mg \cdot L^{-1}$). The same trend was observed for both the $KMnO_4$-treated and control jars. Although NaOCl was not tested in Batch 1 and triple application rate of Simazine was used in Batch 2 as compared to Batch 1 (as phytoplankton remained healthy in Batch 1 with Simazine treatment), we expect a higher endo-MCLR concentration as was recorded in Batch 1, will be accompanied by a higher exo-MCLR concentration after NaOCl or Simazine addition. Thus, improving the aesthetic value of a lake by chemically removing a toxic phytoplankton bloom, could increase potential health risks.

Reglone A or NaOCl application was more effective at removing the mat of phytoplankton than $KMnO_4$ or Simazine application. Addition of Reglone A or NaOCl to a toxic phytoplankton bloom also resulted in a more drastic increase in exo-MCLR than addition of either $KMnO_4$ or Simazine (Fig. 1). The exo-MCLR maximum in the Reglone- and NaOCl-treated jars ranged from 102 ± 2 to 364% and from 64 ± 7 to 152 ± 17%, respectively. In comparison with the Reglone-treated jars, the exo-MCLR maximum was about 2.5- to 4-fold lower in both the $KMnO_4$- and Simazine-treated jars (Fig. 1). Consequently, more MCLR remained inside the Simazine-treated than in the Reglone-treated phytoplankton cells. These findings suggest that Reglone A application may cause more extensive cell damage and lysis than $KMnO_4$ or Simazine application.

Simazine application stops growth and chlorophyll formation by blocking photosynthesis in phytoplankton (Millie et al. 1992). However, the Simazine-treated phytoplankton of Batch 1 remained healthy and there was only a slight increase in exo-MCLR concentrations (22%) compared to the control. I suspect that the shelf-life of the active ingredients of the Simazine used in these experiments had been exceeded. The dosage of Simazine dosage was tripled (*i.e.*, 15 versus 5 $mg \cdot L^{-1}$) in Batch 2 and subsequently the exo-MCLR maxima reached 50 $\pm$ 15% of the initial endo-MCLR concentration. Exo-MCLR concentrations in Batch 2 persisted at greater than 33% of the initial endo-MCLR concentration for 6 days after the chemical addition, indicating that either the toxin was continuously leaking from the cells or Simazine affects toxin degradation. Simazine causes an increase in exo-MCLR concentrations at dosages that remove the phytoplankton scum. Thus, high levels of exo-MCLR would persist in water for at least 1 week following toxic phytoplankton removal by Simazine.

Concentrations of TDP (data not shown), NH_4^+, DOC, and TP (Fig. 3) increased over time after most chemical applications in the Batch experiments. The observed high nutrient concentrations agreed with that expected based on Redfield Ratio of 42:7:1 (the ratio of C:N:P by weight incorporated in phytoplankton tissue). Assuming the carbon content of the freshwater phytoplankton is about 50% of dry-weight (uncorrected for ash; Reynolds 1984), phytoplankton biomass of approximately 1 $g \cdot L^{-1}$ at day 0 was equivalent to 500 mg $C \cdot L^{-1}$. When these phytoplankton decayed and dissociated after chemical addition, based on the Redfield ratio, the concentrations of N and P in the Reglone-treated jars could reach as high as 83 and 12 $mg \cdot L^{-1}$, respectively. In the batch experiments, where Reglone A addition caused the most cell destruction, average NH_4^+ and TP concentrations reached as high as 53 and 8.8 $mg \cdot L^{-1}$, respectively. Trends in NH_4^+ concentrations were similar in all Batches and were probably produced by proteolytic microbes that thrive when proteins become available after the cyanobacterial cell wall weakens (Berg et al. 1987). However, trends in TP and DOC concentrations were not the same in all treatment jars. There were substantially higher TP and DOC concentrations in the Reglone-treated jars than in other chemically-treated jars. In the Reglone-treated jars,

DOC concentration increased by ≥ 35 $mg \cdot L^{-1}$ on day 3 and remined high thereafter. Although 25% of the DOC originated from Reglone A itself, the majority of the DOC however, was likely resulted from the decayed phytoplankton and the released MCLR. In contrast, the DOC and TP concentrations in the $KMnO_4$-treated jars were similar to those in the control jars. TDP followed a similar trend as TP. Thus, nutrients such as TP and DOC are not useful for predicting the concentration of exo-MCLR after chemical addition.

Treatment with alum and lime

In the batch experiments, it was noted that both alum and lime impact phytoplankton through coagulation of cells. The phytoplankton floc sinks to the bottom of the jar leaving a clear water phase after the alum or lime addition. There was almost an immediate clearance of the surface phytoplankton in Batch 3 when higher dosages of both alum and lime (300 and 200 $mg \cdot L^{-1}$, respectively) were used as compared to that in Batches 1 and 2 (200 and 100 $mg \cdot L^{-1}$, respectively). The dosages of lime or alum used in Batch 3 were based on the buffering capacity of lake water. The treated lakewater pH remained at <10 for lime and >6 for alum applications (Fig. 3).

Alum or lime addition to a toxic phytoplankton bloom resulted in minimal increase in exo-MCLR concentrations. Moreover, exo-MCLR concentrations in both the lime- and alum-treated jars were consistently lower than with other treatments (Fig. 1). The addition of lime to toxic phytoplankton resulted in no increase in exo-MCLR concentrations when compared to the control. In contrast, the exo-MCLR concentrations in all the alum-treated jars were up to 3-fold higher (up to 30 $\pm$ 3%) than in the corresponding control jars. The small increase in exo-MCLR concentrations after the addition of alum suggests that aluminum ions might cause cell lysis but to a lesser extent than treatment with Reglone A, NaOCl, $KMnO_4$, or Simazine. Similarly, laboratory experiments have shown that the addition of NaOCl or $KMnO_4$ to an odour-producing strain of *Aph. flos-aquae* resulted in a higher release of the odour compound (geosmin) than that with either alum or lime addition (Peterson et al. in press).

As relatively low exo-MCLR concentrations were detected in both the alum- and lime-treated as well as the control jars, the endo-MCLR concentrations in the alum- or lime-treated phytoplankton were expected to be similar to the control. However, the endo-MCLR concentrations in both the floating alum- and lime-treated phytoplankton were considerably lower than in untreated phytoplankton (Fig. 2 and Table 3), likely due to lime and alum chemically bound to (or precipitating on) the phytoplankton cells (Fig. 4B, C). Thus, when endo-MCLR was expressed as per unit weight, $mg \cdot g^{-1}$ of phytoplankton (dry weight) including attached chemical, the amount of endo-MCLR per unit weight of coagulated phytoplankton was underestimated. For the same reason, the endo-MCLR concentrations in the decaying phytoplankton were also likely underestimated.

The DOC concentrations in both the alum- and lime-treated jars were similar to that in the control (Fig. 3). The TP concentrations in the lime-treated jars were higher relative to the control jars, whereas TP concentrations in the alum-treated jars were lower relative to the control jars (Fig. 3). The exo-MCLR concentrations were consistently lower in the lime-treated jars than in the alum-treated jars, the concentration of TP and DOC in lime-treated jars were higher than in the alum-treated jars for most of the sampling periods. Thus, the change in TP and DOC concentrations did not reflect the exo-MCLR distribution pattern in these two treatments.

SEM and TEM examination

The structure of the chemically-treated *Microcystis* cells was examined by both SEM (Fig. 4) and TEM (Fig. 5). The SEM images showed clearly the chemically precipitated cells whereas the TEM images give a qualitative idea of the extent of cell damage due to chemical addition. A normal *Microcystis* cell has a spherical shape with a smooth exterior (Fig. 4A). *Microcystis* cells that were treated with lime or alum appeared to have a chemical coating on the cell surface (Fig. 4B, C). Those treated with Reglone A or NaOCl were distorted from their normal spherical shape and appeared flattened (Fig. 4D, E). The TEM images of an untreated *Microcystis* cells revealed uniform cell wall with a distinct plasmalemma; cellular organelles such as a nucleus and numerous lipid bodies were also conspicuous (Fig. 5A). Although the cell wall of a lime-treated cell appeared to

have partially separated from the intact plasmalemma, all the cellular contents remained intact (Fig. 5B). Similarly, the TEM image of a alum-treated cell showed a separated but intact plasmalemma and cell wall, together with noticable cellular organelles (Fig. 5C). In contrast, the Reglone-treated cell had an irregular cell wall and has lost its organelles and much of the cytoplasm (Fig. 5D). The NaOCl-treated cell has a convoluted cell wall and indiscernible cellular contents (Fig. 5E). These results suggest that addition of Reglone A or NaOCl to a culture of *M. aeruginosa* caused extensive cell damage and cell distortion whereas lime or alum precipitated cells, caused less cell damage. Thus, the extent of cell damage appeared to correspond with the exo-MCLR concentration in chemically-treated water.

Half-life estimation for exo-MCLR

Reglone A, NaOCl, $KMnO_4$, Simazine, or alum application released exo-MCLR which may be removed by biotransformation and photolysis. Addition of $KMnO_4$, Simazine, or alum caused a gradual increase in exo-MCLR which were not appropriate for computing the half-life of exo-MCLR. Addition of Reglone A or NaOCl however, caused a rapid increase in MCLR in the water phase. The exo-MCLR maximum in the Reglone-treated jars was higher than in the NaOCl-treated jars; there were more data points over the course of exo-MCLR degradation in the Reglone-treated jars (n=3 or 4) than in the NaOCl-treated jars (n=2). Thus, the half-life of exo-MCLR was estimated from the Reglone-treated jars. A plot of $\ln(Ct \cdot Co^{-1})$ versus t yields a straight line, indicating that exo-MCLR followed a first-order exponential decay (Fig. 6). Co (t=0) was estimated from the maximum concentration of MCLR in lake water after the Reglone addition and Ct was the concentration of MCLR at time t. The half-life of MCLR in lake water ranged from 0.5 $\pm$ 0.1 d in Batch 2 to 1.6 $\pm$ 0.0 d in Batch 3 (Fig. 6). At a half-life of 1.6 d, it would take approximately one week for 90% or up to two weeks for 99% of the released toxin to be degraded. The half-life of exo-MCLR estimated from the batch experiments was shorter than the 2.8 d reported by Kenefick et al. (1993) following copper sulfate application to similar batch experiments. As MCLR is structurally stable in water (Wannamacher 1989) and when irradiated with fluorescence light (Tsuji et al. 1994) as in

the present laboratory settings, biotransformation is possibly the main degradation route of MCLR in these batch experiments. The difference in the half-life of MCLR in these batch experiments could result from the difference in microbial densities among batch experiments. Exo-MCLR had a half-life of 10 to 13 d in three *M. aeruginosa* blooms housed in 15-L glass jars without chemical addition (Berg et al. 1987). These results imply that the half-life of naturally occurring exo-MCLR varies with blooms and treatments.

Furthermore, Jones and Orr (1994) showed that the exo-MCLR concentration increased after addition of copper sulfate to a *M. aeruginosa* bloom in Lake Centenary, Australia. Their results suggested exo-MCLR underwent a bi-phasic degradation: a rapid phase of 500 $\mu g \cdot L^{-1} \cdot d^{-1}$ and a slower phase of 10 $\mu g \cdot L^{-1} \cdot d^{-1}$. As biotransformation was suggested to be the degradation route of exo-MCLR in Lake Centenary, the half-life of exo-MCLR in the 'rapid' phase and 'slower' phase would be approximately 0.8 and 4.3 d, respectively. Thus after chemical addition, the half-life of exo-MCLR, estimated from the laboratory and that in Lake Centenary appears to be in the order of a few days.

In contrast to the apparent degradation time for exo-MCLR, endo-MCLR persists within the decaying cyanobacterial cells for a considerable period (*i.e.*, over 26 d or 3.7 week in Batch 3) with only slow degradation. Thus in nature, MCLR may remain at detectable concentrations within the cyanobacteria for a substantial period of time. The persistence of endo-MCLR, together with an over-wintering mechanism, may partly explain the very toxic but sparsely distributed *Microcystis* spp. collected after spring turnover (B. Kotak and A. Lam unpublished data).

Human health implications

Reglone A addition to a microcystin-containing cyanobacterial bloom resulted in a significant release of toxin into the water phase and should therefore not be used to control cyanobacterial-related problems. In addition, the sudden increase in nutrients such as phosphorus and ammonium after Reglone A additions often resulted in a secondary bloom as commonly seen in lakes, thus continuous chemical treatment is required for phytoplankton control. The use of NaOCl as a pre-oxidation chemical in drinking water

treatment also has drawbacks. Chlorination of microcystin-containing cells resulted in an immediate toxin release; however, toxin concentrations subsequently declined to undetectable levels (by HPLC) after 4 d. Further, chlorinated organics can be produced with *M. aeruginosa* acting as an organohalogen precursor (Van Steenderen et al. 1988; Peterson et al. 1993). Both $KMnO_4$- and Simazine-treated cyanobacteria released a lesser amount of MCLR into the surrounding water compared to cyanobacteria treated with Reglone A or NaOCl. The proportion of toxin released after $KMnO_4$ addition was more variable than with Reglone A or NaOCl addition. Simazine additions caused a less drastic but continuous release of microcystin into the surrounding water. Thus, Reglone A, NaOCl, $KMnO_4$ and Simazine do not appear suitable for application to cyanobacterial blooms in drinking water supplies where toxin release may present a problem.

Both alum and lime treatments cause immediate removal (through coagulation and sedimentation) of the floating cyanobacterial layer, leaving a clear water phase. There was no regrowth or resuspension of the cyanobacteria layer within the experimental period (up to 49 d). Both alum and lime additions can also reduce phytoplankton bloom intensity in the subsequent year (Murphy and Prepas 1990). Alum addition resulted in a slight increase in exo-MCLR concentrations possibly due to aluminum ion toxicity to the cyanobacteria. Lime, however, controls the cyanobacterial bloom without a significant increase of exo-MCLR concentrations. For these reasons, lime appears to be an effective chemical for the control of microcystin-containing cyanobacterial blooms in drinking water lakes or reservoirs.

Conclusions

Most microcystin-LR remains in the *Microcystis* cell until the cell is lysed. Cyanobacterial cells are lysed in the presence of chemicals that inhibit new cell wall synthesis, enzymatic reactions, or photosynthesis (*e.g.*, Reglone A, $KMnO_4$, NaOCl, and Simazine). Any sudden release of microcystin into the surrounding water can present a significant hazard to livestock and humans using the water for consumption. Reglone A or Simazine additions result in virtually total release of toxin when used to control

microcystin-containing cyanobacterial blooms. An appropriate waiting period following these application may be necessary before the water is used for consumption. Chlorination and potassium permanganate may not be suitable pre-oxidants in water treatment processes when the water intake is a cyanobacterial-prone source unless the cells are removed prior to the pre-oxidation step. However, both lime and alum treatments (with pH limits used here) appear to control the cyanobacterial bloom mainly by cell-coagulation and sedimentation, with no (lime) or little (alum) increase in exo-MCLR concentration in the water. Lime and alum treatment are thus more favorable than algicides and chlorination for the control of toxic blooms of cyanobacteria.

The persistence of microcystins in aquatic environments is not well documented. The present results show that MCLR degrades exponentially in the water phase with a half-life in the order of a few days. In contrast, endo-MCLR appear to persist within the decaying cyanobacterial cells for weeks (equivalent to 16 MCLR half-lifes or >> 99.9% removal) Thus, it is likely that MCLR would persist and decay inside the alum- or lime-coagulated *Microcystis* cells will likely break down inside the cells before being released into the surrounding water. Further studies on the applicability of these batch experiments to natural lakes and the degradation of MCLR in water are needed.

Table 1. Recommended dosages for lake water and dosages of the six chemicals used in the three Batch experiments ($mg \cdot L^{-1}$). The dosages used in the batch experiments were higher than recommended, as the phytoplankton biomass (as dry weight) was 80-fold more concentrated than in the lake water. 'N/A' indicates the chemical was not used in that Batch experiment.

Chemical	Recommended dosages	Dosages used		
		Batch 1	Batch 2	Batch 3
Reglone	2 - 3.9	20	20	20
NaOCl	0.5 - 1.5	N/A	44	44
$KMnO_4$	1 - 3	10	10	10
Simazine	0.5	5	15	N/A
Alum	132	200	200	300
Lime	25 - 200	100	100	200

Table 2. A summary of the characteristics of control jars for the 3 Batches: dry weight of the bloom material and *Microcystis aeruginosa* biomass on day 0, and the microcystin-LR concentration inside the bloom material on day 0. Microcystin-LR concentrations ($\pm$ *SE*) inside the cells are expressed as $\mu g \cdot g^{-1}$. *M. aeruginosa* biomass is expressed as $cell \cdot mL^{-1}$ and as a percentage of *M. aeruginosa* in the whole phytoplankton community.

Control Jars	Batch 1	Batch 2	Batch 3
Dry weight of bloom material ($g \cdot L^{-1}$)	1.0	1.2 ± 0.1	1.2 ± 0.0
M. aeruginosa ($cells \cdot mL^{-1}$)	4×10^6 (98%)	5×10^5 (66%)	3×10^5 (54%)
Toxin inside cells ($\mu g \cdot g^{-1}$)	837	254 ± 13	182 ± 7

Table 3. Microcystin-LR present in the cyanobacterial bloom (endo-MCLR) and in the water phase (exo-MCLR) 12 d after chemical addition in Batch 2. Endo-MCLR maxima after chemical addition are shown. Both endo- and exo-MCLR concentrations are expressed as a percentage of detectable endo-MCLR (± *SE*) at day 0. 'N/D' indicates non-detectable MCLR and 'N/S' indicates no sample was collected.

Treatment	Endo-MCLR (%)	Exo-MCLR (%)
Control	39 ± 4	11 ± 4
Reglone A	N/D	102 ± 1
NaOCl	8 ± 5	64 ± 7
$KMnO_4$	29 ± 4	13 ± 9
Simazine	N/S	54 ± 11
Alum	9 ± 2	23 ± 15
Lime	25 ± 2	4 ± 0

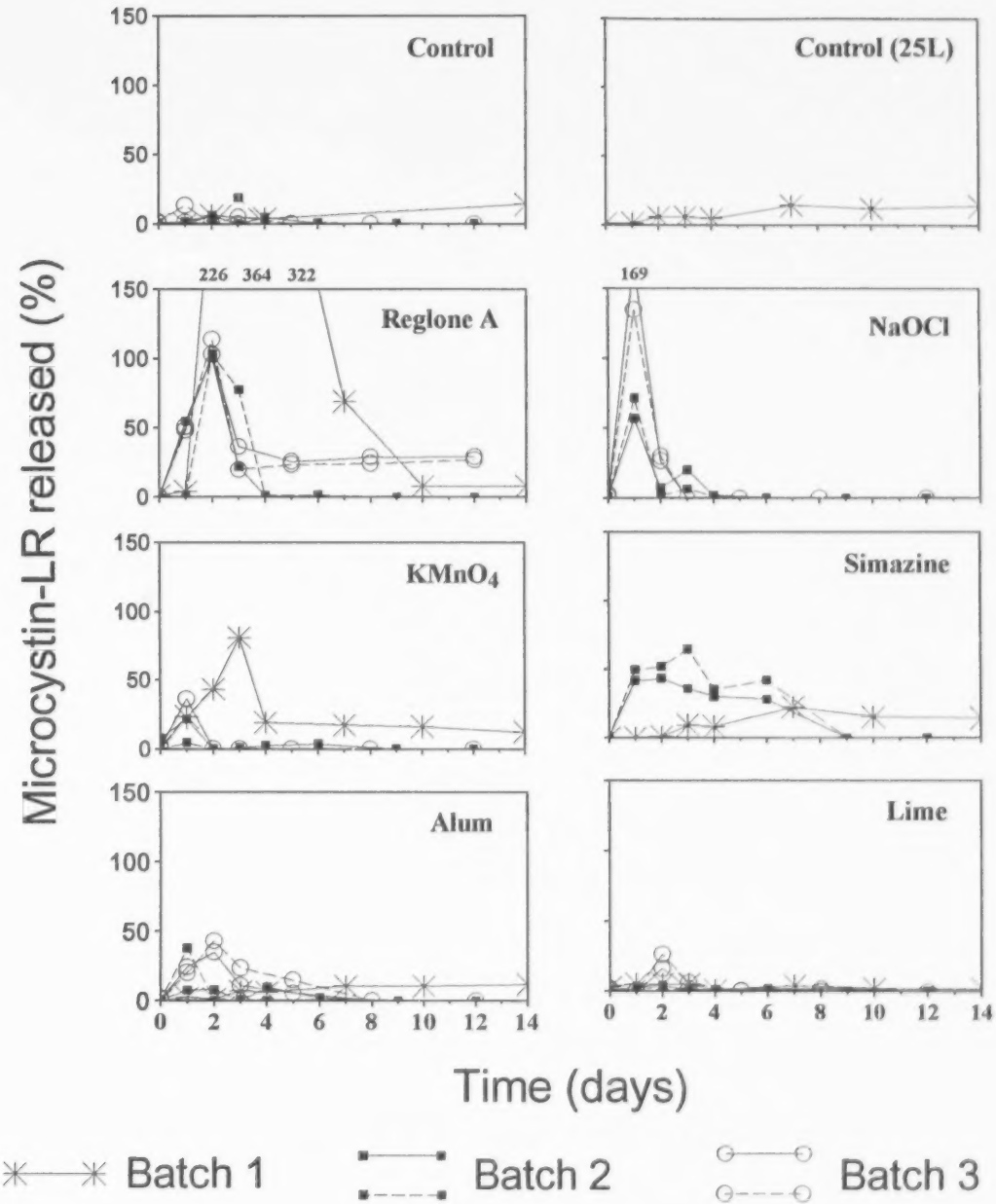

Fig. 1. Release of microcystin-LR (MCLR) into the surrounding water after chemical treatments. The amount released is expressed as a percent of detectable MCLR within the cyanobacterial cells at day 0. Note that a 25-L control was used in Batch 1 since lime treatment was carried out in a 25-L container. Replicates for Batches 2 and 3 are shown.

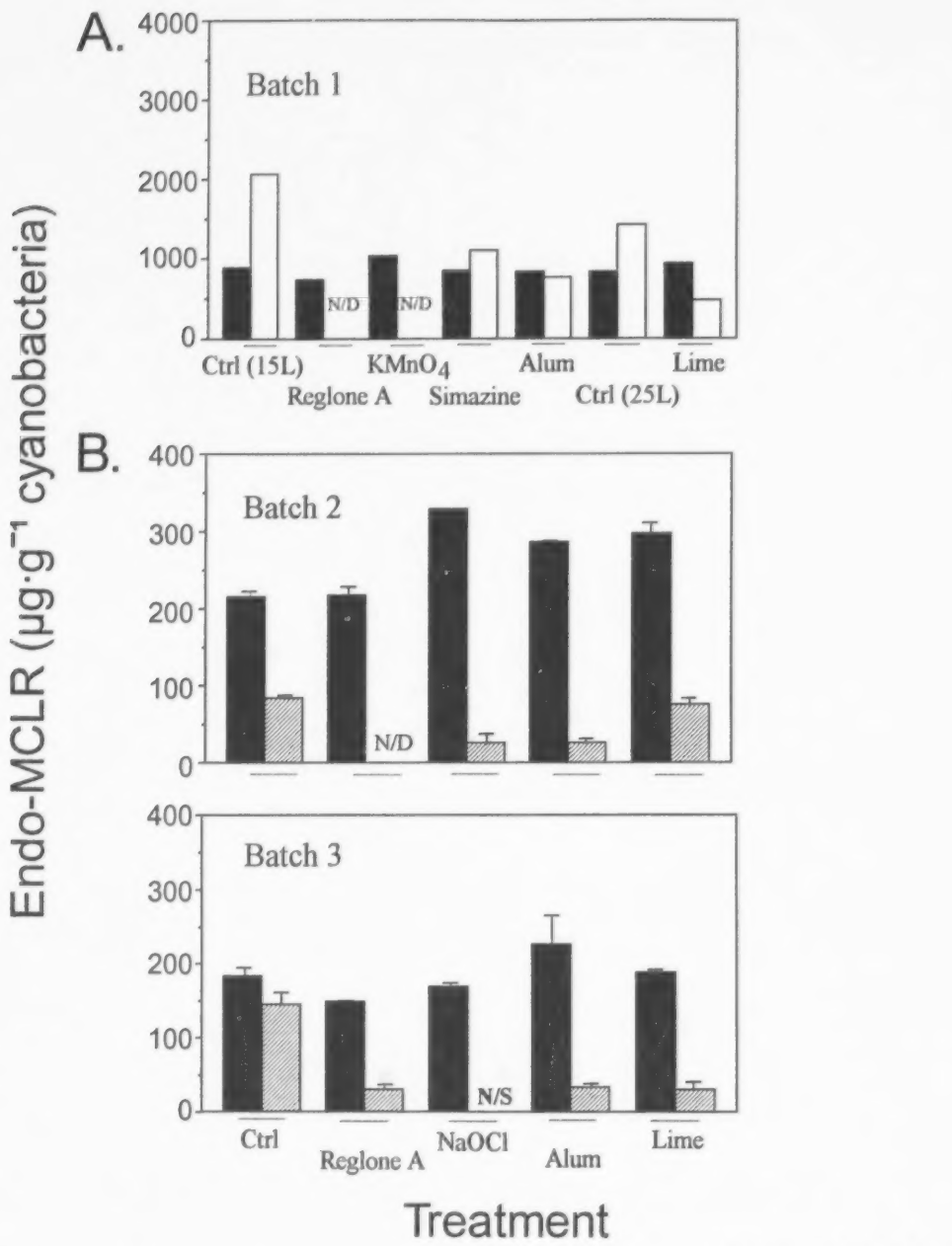

Fig. 2. The amount of MCLR within cyanobacterial cells before and after chemical treatment. The (A) floating and (B) bottom cyanobacterial layers after chemical addition are shown. Note the difference in scales between Batch 1 and Batches 2 and 3.

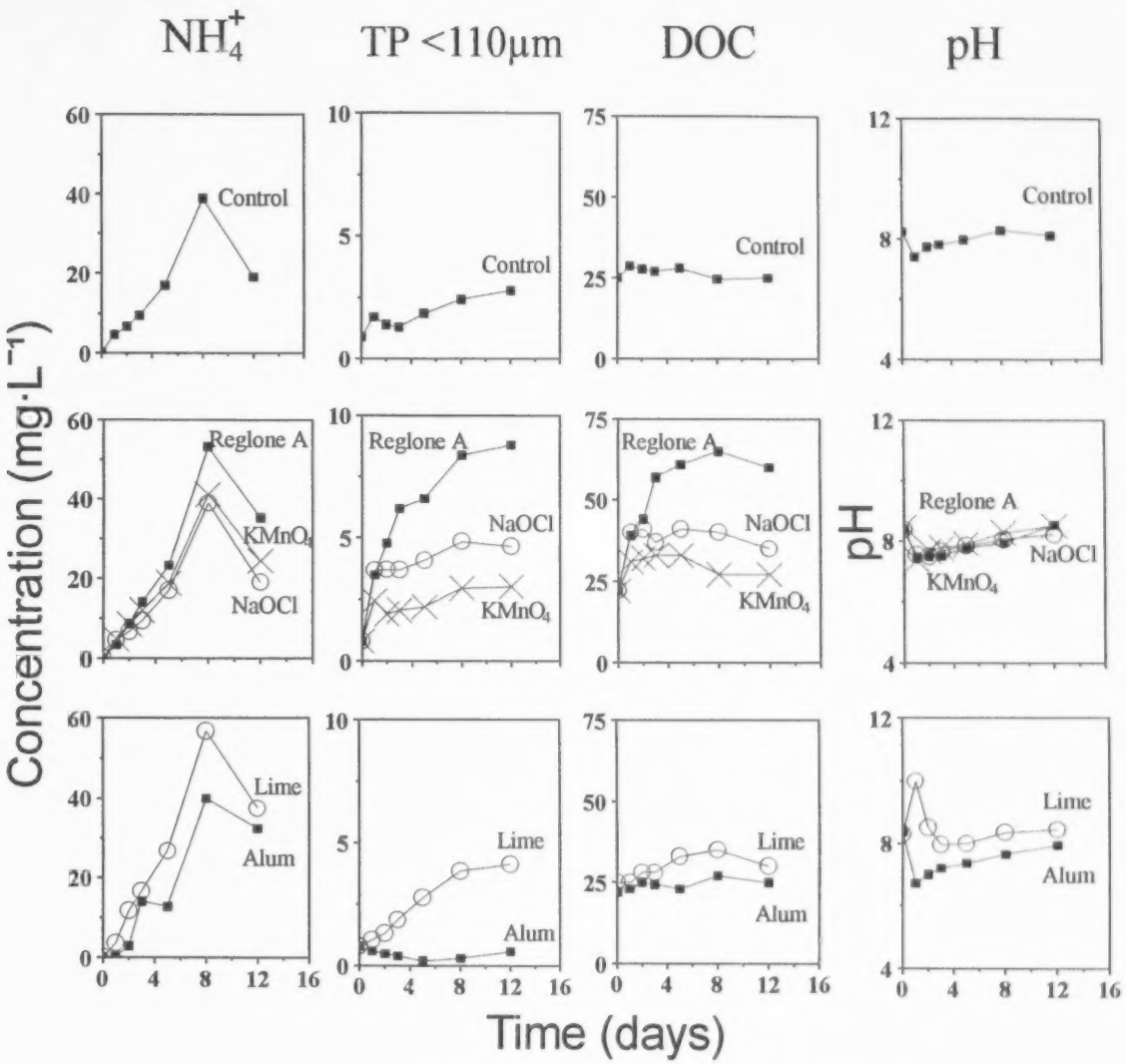

Fig. 3. Mean concentration of NH_4^+, TP (<110 µm), DOC and pH in the water phase of Batch 3 before (day 0) and after chemical treatment.

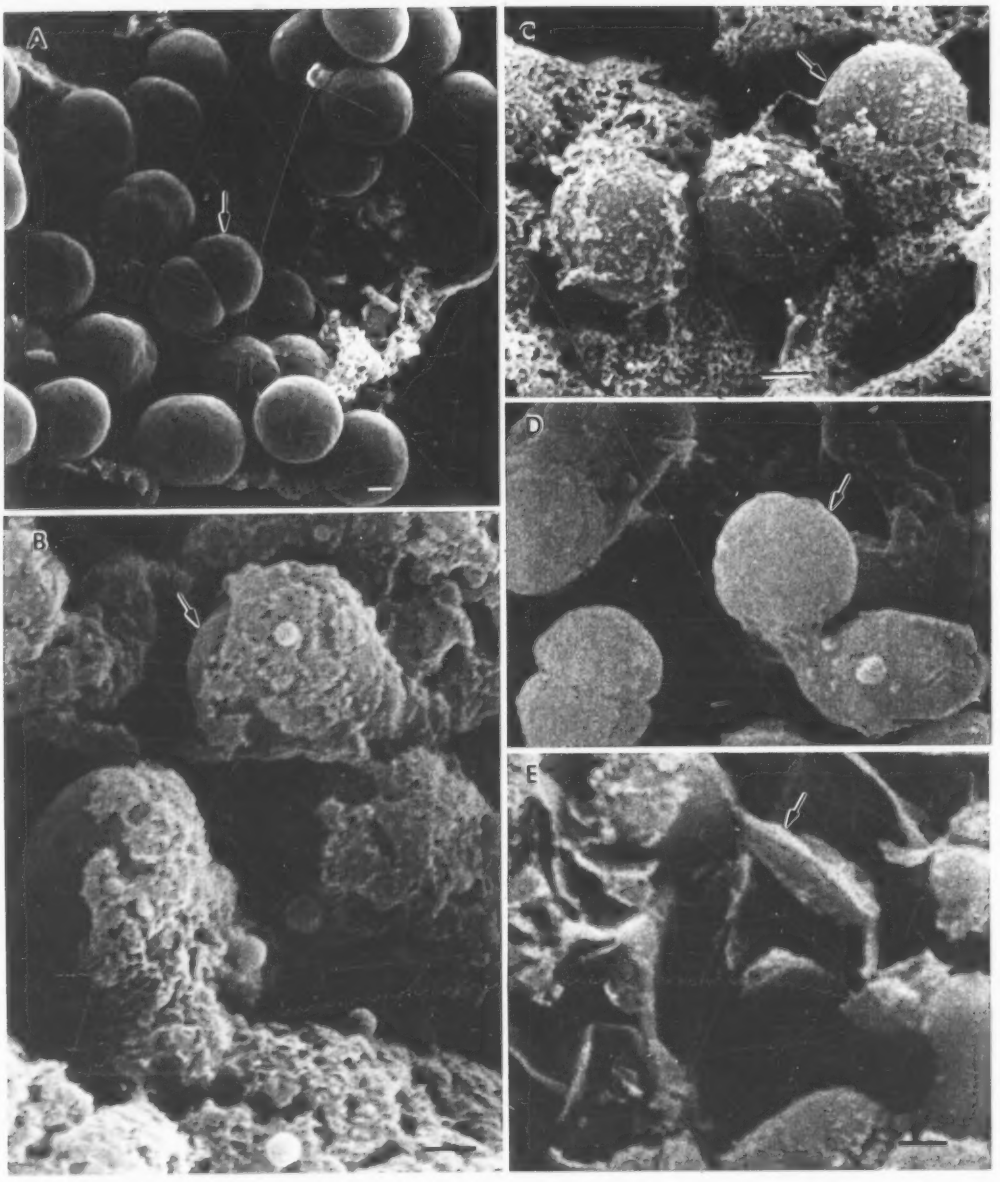

Fig. 4. SEM images of a *Microcystis aeruginosa* culture before (A) and after chemical addition with lime (B), alum (C), Reglone A (D), and NaOCl (E). Scale bars represent 1 μm. Arrows indicate a typical treated *Microcystis* cell.

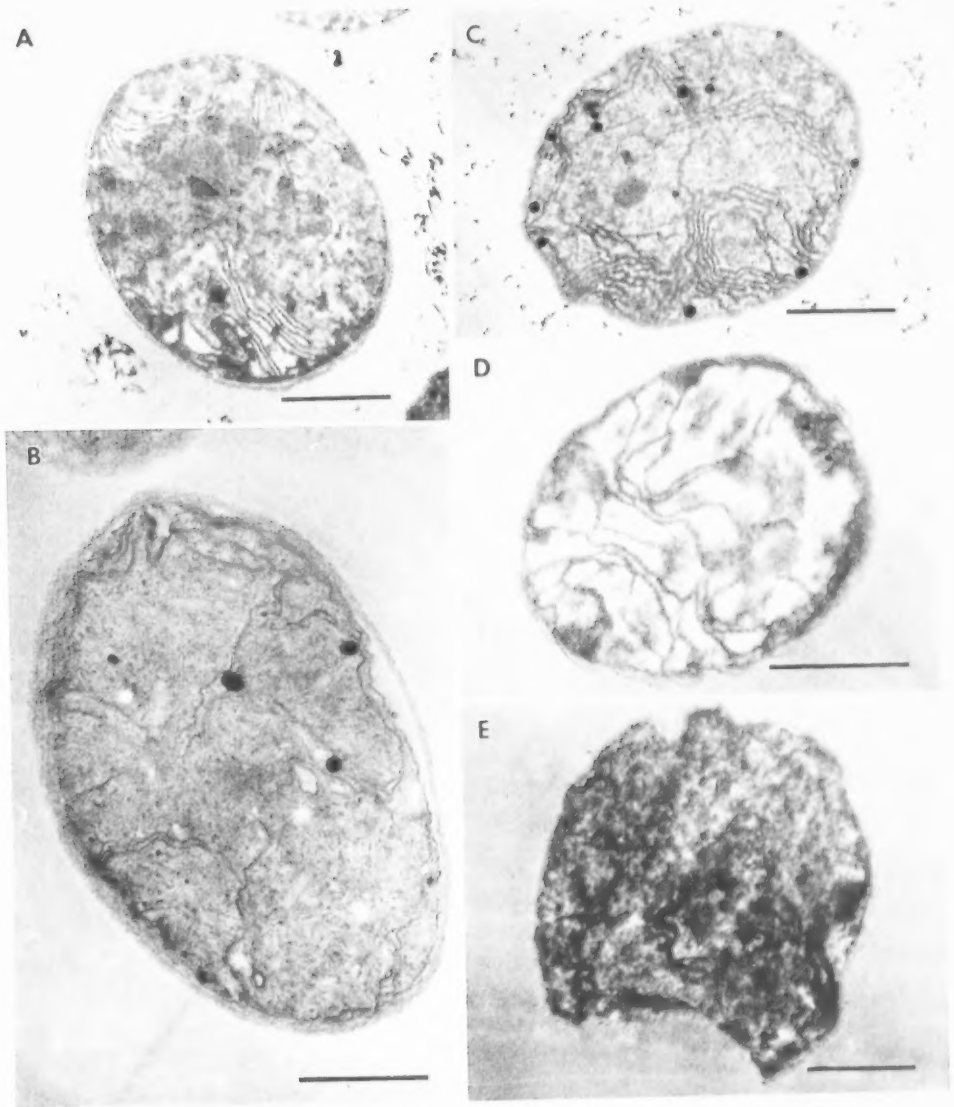

Fig. 5. TEM images of a *Microcystis aeruginosa* culture before (A) and after chemical addition with lime (B), alum (C), Reglone A (D), and NaOCl (E). Scale bars represent 1 μm. Note the lime and alum-coating on the cell membrane in (B) and (C), respectively.

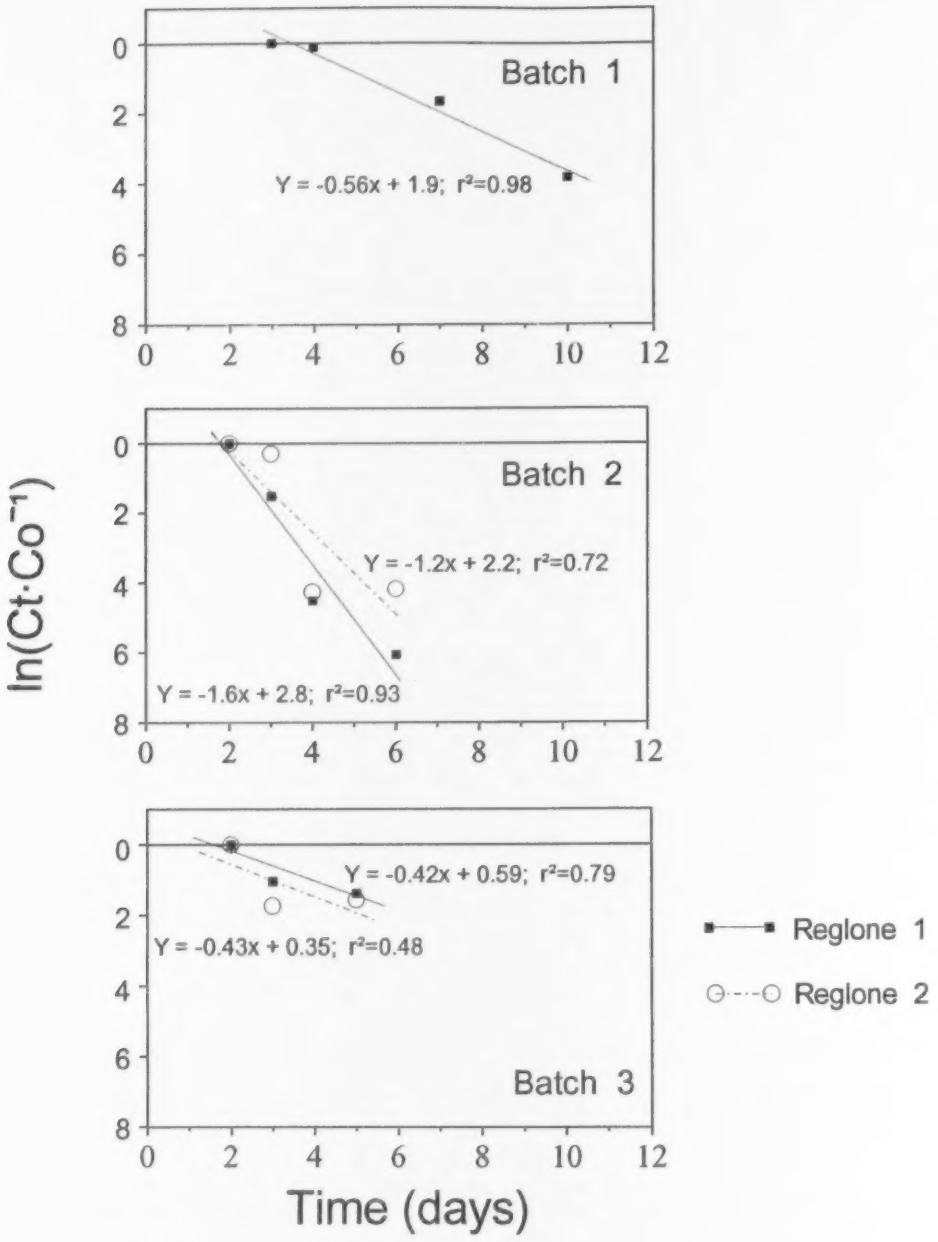

Fig. 6. A straight line plot of $\ln(Ct \cdot Co^{-1})$ versus t indicated that MCLR followed a first-order exponential decay. Co (t=0) was estimated from the maximum concentration of MCLR in lake water after the Reglone addition and Ct was the concentration of MCLR at time t. The half-life of MCLR in lake water is 1.25 days (n=1), 0.52 $\pm$ 0.07 days and 1.62 $\pm$ 0.03 days for Batches 1, 2, and 3, respectively.

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IV. In-situ evaluation of options for chemical treatment of hepatotoxic cyanobacterial blooms[5]

Introduction

Cyanobacteria of the genera *Microcystis*, *Oscillatoria*, and *Anabaena* are known to produce a number of secondary metabolites, some which cause taste and odour problems in drinking water supplies (Matsumoto and Tsuchiya 1988). Recently, concern has grown in the water treatment industry about potential contamination of drinking water supplies by toxic metabolites called microcystins, a family of at least 50 tumor-promoting hepatotoxins (Carmichael 1994). There is no apparent relationship between the production of microcystins and odorous secondary metabolites by *Microcystis aeruginosa* (Kenefick et al. 1992; Hrudey et al. 1993).

Microcystins are small cyclic peptides that classically consist of five largely 'invariable' amino acids (D-alanine, erythro-β-methylaspartic (MAsp), D-glutamic acid (Glu), N-methyldehydro-alanine (Mdha), and 3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl-deca-4,6-dienoic acid (Adda)), and two 'variable' L-amino acids (Fig. 1). The LD_{50} of the most commonly reported microcystin, microcystin-LR (MCLR), is 50 $\mu g \cdot kg^{-1}$ in mice (by intraperitoneal injection). Based on a conversion factor of 62 times from intraperitoneal injection to oral dose (Falconer et al. 1988), the oral LD_{50} of MCLR would be 3100 $\mu g \cdot kg^{-1}$ in mice. Like toxins linked to incidents of diarrhetic shellfish poisoning (*i.e.*, okadaic acid), microcystins specifically inhibit protein phosphatase-1 and -2A (PP-1 and PP-2A, respectively), two of the major serine/threonine protein phosphatases which regulate cellular metabolism and cell division in eukaryotic cells (Cohen et al. 1990). Microcystin, as a hepatotoxin, induces chronic liver damage when exposure is at sublethal dosages (Falconer et al. 1983, 1988; Carmichael and Falconer 1993).

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5. A version of this chapter has been submitted for publication. A. K.-Y. Lam and E. E. Prepas. *Can. J. Fish. Aquat. Sci.*

Microcystins are found mostly in fresh water and to a lesser extent in brackish water. The release of intracellular microcystin (endo-MCYST) from toxic cyanobacteria occurs during cell aging (Berg et al. 1987). Dramatic release (as high as 1800 $\mu g \cdot L^{-1}$) of endo-MCYST has been reported in lake water when cyanobacteria were treated with copper sulfate (Jones and Orr 1994). Dissolved microcystins in the water phase (exo-MCYST) are likely to have a direct impact on human health when they are present in drinking water supply lakes. To understand the health risks associated with exo-MCYST, knowledge of the persistence of exo-MCYST in lake water and the effect of different chemical treatments on the release of endo-MCYST from cyanobacteria is needed.

The four primary degradation routes for exo-MCYST in lake water are hydrolysis, photolysis, biodegradation/biotransformation, and sorption. Exo-MCLR undergoes only slow degradation in water (0.37% per day at 45°C; Wannemacher 1989). Photochemical decomposition of MCLR was observed only in cyanobacterial pigment solutions (> 0.005 $g \cdot L^{-1}$; pigment concentration was based on weight of freeze-dried cyanobacteria$\cdot L^{-1}$) when irradiated with natural sunlight; the photodecomposition rate of MCLR increased with increasing pigment concentration. However, MCLR did not photodecompose when irradiated with fluorescent light (Tsuji et al. 1994). Biotransformation of MCLR to non-toxic product(s) was detected after an initial lag period of 2-10 d (Jones and Orr 1994; Jones et al. in press; Lam et al. in press a); the biotransformation followed first-order kinetics with a half-life <3.6 d (Kenefick et al. 1993; Lam et al. in press a, b). No study has yet explored the ability of MCLR to sorb to lake sediments. Toxicants known to sorb to lake sediments can persist there for over > 30 yr (Miskimmin and Schindler 1994). Thus, the sorption capability of microcystin could be an important consideration when determining its persistence in lake.

The effects of chemical treatments on microcystin release from cyanobacteria have been studied primarily in laboratory experiments with freshly collected phytoplankton. Lam et al. (in press b) categorized chemical treatments on the basis of their effect on MCLR release. Group 1 chemicals disrupt cell functions and induce cell lysis; they include copper sulfate (Kenefick et al. 1993), Reglone A, chlorine, and potassium permanganate

(Lam et al. in press b). Group 2 chemicals precipitate (or coagulate) phytoplankton cells and leave cells essentially intact; they include lime ($Ca(OH)_2$; Kenefick et al. 1993; Lam et al. in press b) and alum ($(Al_2SO_4)_3 \cdot 14H_2O$); Lam et al. in press b). Group 2 chemicals were recommended for treating toxic phytoplankton blooms because they induce minimal release of MCLR into the surrounding water (Lam et al. in press b). Increased microcystin concentrations in lake water have been reported after in situ copper sulfate addition to toxic blooms (Jones and Orr 1994); however, the impact of other chemical treatments on toxin release from cyanobacterial blooms in a lake setting has not been studied.

A whole-lake study in a lake used as a drinking water supply, on the persistence of exo-MCYST after chemical addition poses potential human health risks. At the same time, results generated from laboratory experiments with highly concentrated bloom material or purified MCLR may not be applicable to a lake setting. Limnocorrals in lakes represent a compromise between these two approaches as the exo-MCYST released would not contact the main body of lake water and pose health risks to nearby residents. Limnocorrals also allow the inclusion of sunlight, natural populations of bacteria, and an environmentally relevant concentrations of toxic phytoplankton. Thus, limnocorrals can be used to examine the applicability of laboratory experiments to a more natural setting.

In this two-part study, I (1) determined the partitioning of MCLR between water and natural lake sediments (sorption), and (2) did two in-situ experiments with closed-bottom limnocorrals to: (a) evaluate the suitability of Reglone A (Group 1 representative) and lime/alum (Group 2 representative) for the control of hepatotoxic blooms of cyanobacteria; and (b) determine the persistence of exo-MCYST in lake water following chemical treatment in limnocorrals.

Materials and Methods

Sorption. Little Beaver, Driedmeat, and Coal are hardwater lakes located in the Battle River drainage basin in Alberta, Canada. These lakes have been monitored since 1990 as part of a study on the occurrence and seasonal dynamics of toxic phytoplankton in

lakes. These three lakes have similar morphometry and water quality (Mitchell and Prepas 1990), and are microcystin-prone (Kotak et al. 1993; submitted).

Surface sediments were collected with an Ekman dredge on 26 and 27 May 1992 from all three lakes. The sediments were sterilized at 120°C for two 30-min cycles, freeze-dried and then analysed for organic content (difference between freeze-dried and ashed sediment weight). Sediments in Little Beaver Lake (26% by weight) had a higher organic content than Driedmeat or Coal lakes (21 and 12%, respectively). Karickhoff et al. (1979) showed that the partition coefficient of toxicants was a positive function of the organic content of the sediment, therefore Little Beaver Lake sediments were chosen to represent the 'worst-case scenario' among the three lakes. Little Beaver Lake sediments were further characterized by water content (difference between air-dried and freeze-dried sediment weight) and composition (Table 1).

Autoclaved double distilled water (60 mL) with 100 µL of 40% formalin was placed in each of the nine 125-mL Erlenmeyer flasks. Each treatment was done in triplicate. Purified MCLR was added to each flask to give an initial concentration of 100 $\mu g \cdot L^{-1}$. Two sediment concentrations, 5 and 10 g sediments per liter double distilled water, represent concentrations at the water-sediment interface where sorption is most likely to occur; controls flasks had MCLR but no sediment. Three sets of samples (a total of 27 flasks) were capped with foam plugs, and shaken continuously in the dark at 24 $\pm$ 1 °C for 48 h. Flasks were chosen randomly at 0, 24 and 48 h after exposure to sediments for MCLR analysis. Sediment samples were centrifuged, and MCLR remaining in the water phase was analyzed by reversed-phase high performance liquid chromatography (HPLC) as outlined by Kenefick et al. (1992) with the following modification: the toxin was eluted from the C-18 cartridge in 1 mL HPLC solvent and an injection volume of 75 µL was used. A two-way analysis of variance (Wilkinson et al. 1992) was used to evaluate the sorption capability of MCLR.

Chemical treatment in limnocorrals. A toxic phytoplankton bloom ($\geq$ 2 $mg \cdot L^{-1}$ phytoplankton biomass at 25 µg MCYST$\cdot g^{-1}$ biomass; dry weight) was needed to provide a detectable supply of exo-MCYST during these experiments. Experiments were

conducted in Driedmeat Lake on 19 July and 16 August 1993 (Experiments 1 and 2, respectively) in closed-bottomed limnocorrals because: 1) cyanobacterial bloom density and microcystin concentrations were higher than in either Little Beaver or Coal lakes on those dates, 2) MCLR concentration varied among the study lakes and from year-to-year (Kotak et al. submitted) hence, limnocorrals allowed for a flexible experimental location, and 3) results of the sorption experiments indicated that contact with lake sediment was not necessary to understand microcystin dynamics in lake water. Based on the above considerations, the persistence of microcystin in a closed-bottomed limnocorral should be applicable to a natural lake setting.

All limnocorrals were 1.5 m diameter by 1.5 m high and held 2650 L of water. They were made of clear woven polyethylene fitted with a flotation collar of ethafoam logs (10-cm diameter). To keep each limnocorral vertical in the water column, a heavy metal chain was attached around the bottom edge. Each limnocorral was then secured to a plastic frame which was anchored to a dock, which also served as a base for sample collection.

Experiment 1. Lake water was pumped from a depth of approximately 0.5 m into the limnocorrals. Extra phytoplankton were collected with a conical 64-μm mesh net (30 cm diameter by 100 cm length) fitted with a brass bucket; phytoplankton were added in equal amounts to a total of six limnocorrals. The limnocorrals were left to acclimatize for 2 d before chemical addition. Experiment 1 was done in duplicate with a control and two chemical treatments. Reglone A (15 $\mu L \cdot L^{-1}$ or 22 $L \cdot ha^{-1}$ or 3 μg diquat$\cdot L^{-1}$) represented chemicals that induce cell lysis, and hydrated lime (100 $mg \cdot L^{-1}$) represented chemicals that precipitate (or coagulate) cyanobacterial cells (Lam et al. in press b). Two sites in the lake near the limnocorral area were used as reference sites for evaluation of differences between water inside and outside of the limnocorrals. At the end of Experiment 1, all plastic-framed limnocorrals were removed from the lake, brushed and washed with tap water, and used in Experiment 2.

Experiment 2. A 30-cm tall splash guard made of plastic sheet supported by plastic pipe was installed perpendicular to each of the plastic frames to prevent lake water from entering the limnocorrals (as occurred in Experiment 1). To dampen wave action, logs (about 20 cm in diameter) were anchored approximately 2 m from the experimental area

on the windward sides. Lake water from approximately 0.5 m depth was pumped into each limnocorral and left to acclimatize for 2 d before chemical addition.

Experiment 2 was done in triplicate. The Reglone-treated limnocorrals were treated at days 0 (3 μg diquat·L^{-1}), 2 (1.5 μg diquat·L^{-1}) and 3 (3 μg diquat·L^{-1}). The lime-treated limnocorrals were also treated with alum to maintain a pH between 7 and 10, since the water in the limnocorral was separated from the natural buffering capacity of the bottom sediments. A mixture of 175/175 mg·L^{-1} lime/alum (Babin et al. 1992) was added to the limnocorrals on day 0, and a mixture of 100/50 mg·L^{-1} lime/alum was added again on day 4. Three limnocorrals with no chemical treatment were used as controls, for a total of nine limnocorrals. Three reference sites, a distance of approximately 2 m from the limnocorral area, were sampled as well.

Integrated water samples (surface to a depth of 0.75 m) were collected from each limnocorral and reference site with weighted Tygon tubing (I.D. = 1.6 cm) fitted with a one-way valve. Water samples were filtered through Whatman GF/C filters and then stored at -20 °C. Exo-MCYST was analysed by protein phosphatase (PP) assay (Holmes 1990; Lambert et al. 1994) using ^{32}P-radiolabelled phosphatase *a* as the substrate and PP-1c as the enzyme. The PP assay was used because it detects total microcystin concentrations; this is more relevant to health risk assessment than MCLR alone as cyanobacteria are known to produce several microcystin analogues (Watanabe et al. 1989; Craig et al. 1993) concurrently with no clear pattern of analogue distribution (Watanabe et al. 1989). As the PP assay does not distinguish among microcystin analogues, the results of the PP assay is thus presented as micrograms of MCLR equivalent per liter lake water. The detection limit of PP assay in this study was 0.05 μg·L^{-1}. Total phosphorus (TP) and total dissolved phosphorus (TDP; ≤ 0.45μm) were determined by the persulfate digestion method (Menzel and Corwin 1965) as modified by Prepas and Rigler (1982). Ammonium (NH_4^+; after Solorzano 1969) and nitrate+nitrite (NO_3^-; Stainton et al. 1977) were determined on a Technicon AutoAnalyzer. Potassium ion (K^+) concentration was measured by atomic absorption (Perkin-Elmer Model 3030). Water samples for phytoplankton enumeration were preserved with Lugol's solution; enumeration was

performed with a Carl Zeiss Sedival Hydrobiological Inverted Microscope by the sedimentation method (Utermöhl 1958). Chlorophyll *a* (Chl*a*) was analysed by ethanol extraction (Bergmann and Peters 1980). Chl*a* concentration was used to represent the intensity of the phytoplankton bloom.

Phytoplankton were collected for toxin analysis with a 64-µm mesh conical net (18 cm diameter by 38 cm length) which was hauled vertically through the water column from a depth of 0.75 m to the water surface; the number of hauls was recorded for estimation phytoplankton biomass dry weight per liter of lake water. Freeze-dried phytoplankton samples were extracted twice with 5% acetic acid as described by Kenefick et al. (1992), and analysed by PP assay (Holmes 1990) for endo-MCYST concentrations for all sampling dates. Intracellular microcystin-LR (MCLR) concentration on day 0 was measured by reversed-phase HPLC.

Experiments 1 and 2 were intended to run for 14 d but only lasted for 4 and 7 d, respectively, due to wave damage to the limnocorrals. Samples were collected immediately before (day 0) and after chemical addition on days 1, 2, 3, 4 and 7. Three limnocorrals in Experiment 1 developed tears at the joint of the flotation collar between days 2 (lime-treated and control) and 3 (Reglone-treated). Consequently, the results of Experiment 1 will only be discussed qualitatively.

Repeated measures on analysis of variance (P <0.05) were used to compare exo-MCYST concentrations in Experiment 2 (Wilkinson et al. 1992). Correlation analysis was used to examine the relationships between endo- and exo-MCYST concentrations and between exo-MCYST and other nutrients in lake water (Wilkinson et al. 1992).

Results

Sorption. The water content of surface sediments from Little Beaver Lake was 89%. Freeze-dried sediments had a 26% organic content. Based on Wentworth size class distribution, Little Beaver Lake sediments were comprised mainly of clay and silt (52 and 48%, respectively; Table 1). When purified MCLR was added to sterilized, freeze-dried lake sediments, no significant reduction (P >0.99) was recorded in dissolved MCLR

concentrations in the water phase of both the 5 and 10 $g \cdot L^{-1}$ sorbent concentrations over the 24- and 48-h periods (Fig.2). Thus, if decomposition of exo-MCYST occurred, it would likely be via biotransformation and/or photolysis which would be in the water column of a lake.

Chemical treatment in limnocorrals.

Experiment 1. Prior to treatment (day 0), mean chl*a* concentrations in the control, lime- and Reglone-treated limnocorrals and at the reference site were similar (29, 26, 22, and 20 $\mu g \cdot L^{-1}$, respectively). The dominant phytoplankton was the cyanobacterium *Aphanizomenon flos-aquae* (80 to 90% by volume) while *Microcystis aeruginosa* made up <3% (or 0.5 $mm^3 \cdot L^{-1}$) of the phytoplankton community. The average pH of the lake water was 9.2 and exo-MCYST concentrations were below detection in the limnocorrals. The pH of the water in the lime-treated limnocorrals increased to 10.8 immediately after lime addition and remained ≥ 10 for the following 3 d. No detectable change in the pH of the water was noted in the control or Reglone-treated limnocorrals from days 0 to 1. Chl*a* concentrations decreased substantially in the Reglone-treated and lime-treated limnocorrals (82 to 79%, respectively), decreased slightly in the controls (7%), and increased by 50% at the reference sites. Concomitant with the decreased chl*a* concentrations in the limnocorrals, exo-MCYST concentrations increased from below detection (<0.05 $\mu g \cdot L^{-1}$) to maximum concentrations of 0.32 and 0.42 $\mu g \cdot L^{-1}$ in the lime- and Reglone-treated limnocorrals, respectively. Exo-MCYST concentrations changed minimally both in the control limnocorral and at the reference site over the 4-day experimental period. Thus, addition of lime (pH >10) or Reglone A removed cyanobacteria but caused a concomitant increase in exo-MCYST concentrations.

Experiment 2. Increase in microcystin in the lime-treated limnocorral in Experiment 1 was likely caused by the sudden pH shock after lime addition. To maintain pH 7-10 in lake water with reduced buffering capacity, a mixture of lime and alum was used (Babin et al. 1992). Post-treatment pH in the limnocorrals and at the reference sites were similar (9.6 and 9.3, respectively).

Pre-treatment characteristics of all the limnocorrals and reference sites are summarized in Table 2. Chl*a* concentrations in the control, lime/alum-treated, and Reglone-treated limnocorrals were 2.4 times higher than in Experiment 1. At the reference sites, chl*a* concentrations and phytoplankton volumes were 2 to 3 times lower than in the limnocorrals. The phytoplankton community was co-dominated by *Aph. flos-aquae* and *M. aeruginosa*, which collectively constituted over 95% of the phytoplankton community by volume in both the limnocorrals and reference sites. The relative proportion of *M. aeruginosa* by volume among the limnocorrals was similar (22-37%), but was 2 to 3 times higher at the reference sites; average cell volume of *M. aeruginosa* ranged from 8.5 (reference) to 16 $mm^3 \cdot L^{-1}$ (control). Other minor species (≤ 5%) present in the bloom were: *Anabaena flos-aquae*, *Merismopodia* sp., *Phormidium mucicola*, *Rhodomonas minuta*, and *Clamydomonas* sp.

Endo-MCYST concentrations were similar among limnocorrals (0.48 to 0.51 ± 0.04 $\mu g \cdot g^{-1}$), and nearly 3-fold higher at the reference sites (1.4 ± 0.08 $\mu g \cdot g^{-1}$ biomass). Endo-MCLR concentrations at day 0, as measured by HPLC, was consistently lower than endo-MCYST concentrations. Specifically, average endo-MCLR concentrations comprised 32, 27, 36, and 48% of the endo-MCYST concentrations in the control, lime/alum-, and Reglone-treated limnocorrals, and the reference site, respectively. When endo-MCYST concentrations were expressed in μg MCLR equivalent$\cdot L^{-1}$, they ranged from 0.66 $\mu g \cdot L^{-1}$ in the control limnocorrals to 1.52 $\mu g \cdot L^{-1}$ at the reference sites (Table 2). Exo-MCYST concentrations were however similar (0.25-0.33 $\mu g \cdot L^{-1}$; Table 2), particularly given the almost 2-fold difference in *Microcystis* cell volume between the limnocorrals and reference sites (Table 3). This suggested that the integrity of *Microcystis* cells was similar among all limnocorrals and at the reference sites at the beginning of Experiment 2.

Over the course of the 7-d experiment, chl*a* concentrations were correlated with phytoplankton volumes in the control, lime/alum-, and Reglone-treated limnocorrals (r=0.86, 0.97, and 0.84; n=18, 17, and 17, respectively; $P<<0.001$) and at the reference sites (r=0.71; n=18; $P<0.001$). By day 7, mean chl*a* concentrations increased by 14% in

the control limnocorrals and by 150% at the reference sites relative to day 0 (Fig. 3), primarily due to an increase in *Aph. flos-aquae* cell volume (Table 3). Post-treatment chl*a* concentrations in both the lime/alum- and Reglone-treated limnocorrals dropped to <5 $\mu g \cdot L^{-1}$ (a 20-fold decrease; Fig. 3). Chl*a* concentrations were correlated with phytoplankton volumes in both the lime/alum- and Reglone-treated limnocorrals (r=0.97 and 0.84, respectively; n=17; P<<0.001). Post-treatment phytoplankton concentrations in the Reglone-treated limnocorrals were lower than in the control limnocorrals; however, the post-treatment *Microcystis* volume was unexpectedly higher in the Reglone-treated limnocorrals than in the control limnocorrals (Table 3). When examined under the inverted microscope, the *Microcystis* cells in the Reglone-treated limnocorrals did not have a clear cell structure and were probably 'leaky' cells that were resuspended into the water column due to wave action. Thus, lime/alum treatment appeared to sediment *Microcystis* cells whereas Reglone A treatment did not.

Despite the 2-fold increase in phytoplankton volume (mainly *Aph. flos-aquae*) at the reference sites, no correlation was noted between phytoplankton volume and endo- or exo-MCYST concentration (P>0.2). At the reference sites, post-treatment endo- (1.29-1.83 $\mu g \cdot L^{-1}$) and exo-MCYST concentrations (non-detectable to 0.16 $\mu g \cdot L^{-1}$) fluctuated over the 7-d (Fig. 4). Trends in both endo- and exo-MCYST concentrations were similar between the control limnocorrals and the reference sites. In the control limnocorrals, post-treatment concentrations of endo-MCYST remained consistantly higher than exo-MCYST (ranged from 0.25 to 0.87 $\mu g \cdot L^{-1}$ and non-detectable to 0.05 $\mu g \cdot L^{-1}$, respectively). Thus, most microcystin was retained inside cells in both the control limnocorrals and reference sites.

In the lime/alum-treated limnocorrals however, post-treatment endo-MCYST concentrations decreased to below detection on day 1 (a 21-fold decrease), remained the same from days 2 to 4, and increased to 0.38 $\mu g \cdot L^{-1}$ on day 7 (Fig. 4). Pre-treatment exo-MCYST concentrations in the lime/alum-treated limnocorrals decreased from 0.33 ± 0.00 $\mu g \cdot L^{-1}$ on day 0 to the detection limit on day 1, remained at a mean of 0.10 $\mu g \cdot L^{-1}$ between day 2 and 3, and were below detection thereafter. Endo-MCYST concentrations were

negatively correlated with exo-MCYST only in the Reglone-treated limnocorrals (r= -0.58; n=17; P<0.02; Fig. 4). In the Reglone-treated limnocorrals, average endo-MCYST concentrations declined gradually from 1.1 $\mu g \cdot L^{-1}$ on day 0 to below detection on day 4, and increased to 0.19 $\mu g \cdot L^{-1}$ on day 7. Meanwhile exo-MCYST concentrations in the Reglone-treated limnocorrals increased by 11-fold to a maximum of 3.7 $\mu g \cdot L^{-1}$ on day 2, and remained at high concentrations (ranging from 2.2 ± 1.1 to 3.4 ± 0.07 $\mu g \cdot L^{-1}$) for the next 5 d. Maximum exo-MCYST concentrations in the Reglone-treated limnocorrals was about 330% higher than the initial extractable endo-MCYST. When Reglone A alone was added to PP-1c, no inhibition effect was detected. This result suggested that many of the hydrophobic microcystins were not extractable by the C-18 cartridge plus acetic acid extraction method; thus, total endo-MCYST could likely be underestimated when this extraction method is used. Exo-MCYST concentrations in the Reglone-treated limnocorrals were on average 29 times higher than those in the lime/alum-treated limnocorrals, whereas the highest detectable exo-MCYST concentration in the lime/alum-treated limnocorrals (0.11 $\mu g \cdot L^{-1}$) was similar to that at the reference sites (0.16 $\mu g \cdot L^{-1}$).

Average TP and TDP concentrations did not change appreciably (≤ 5%) in the control limnocorrals over the 7-d period (Fig. 5). At the reference sites, increase in TDP and TP concentrations coincided with increased phytoplankton volumes (r=0.66 and 0.73, respectively; n=18; P<0.005). In the lime/alum-treated limnocorrals, both TDP and TP concentrations decreased to 45 and 114 $\mu g \cdot L^{-1}$ (a 6-fold decrease) on day 1, respectively, which coincided with a similar decrease in phytoplankton volumes throughout (r=0.84 and 0.63, respectively; n=17; P<0.007). However, in the Reglone-treated limnocorrals, both TDP and TP concentrations were negatively correlated with phytoplankton volumes (r= -0.86 and -0.66, respectively; n=17; P<0.005). There was no difference between TDP and TP concentrations in the Reglone-treated limnocorrals (n=12; P>0.05), thus most of the P in the Reglone-treated limnocorrals was smaller than 0.45 µm. Both TP and TDP were positively correlated with exo-MCYST in the Reglone-treated limnocorrals (r=0.72 and 0.70, respectively; n=17; P<0.002); however, TP was weakly correlated with exo-MCYST concentrations at the reference sites (r= -0.50; n=18; P<0.05). No correlation

between P and exo-MCYST was observed in the control and lime/alum-treated limnocorrals.

No clear pattern was seen in dissolved inorganic nitrogen (DIN) concentrations in either the limnocorrals or at the reference sites over the 7-d experiment (Fig. 6). Except for the sudden increase in NH_4^+ concentrations on day 3 in one of the controls, average DIN concentration over the 7-d experiment was lowest in the reference sites (9.3 and 1.2 $\mu g \cdot L^{-1}$ for NH_4^+ and NO_3^-, respectively), followed by the control (43 and 1.8 $\mu g \cdot L^{-1}$) and lime/alum-treated limnocorrals (68 and 2.8 $\mu g \cdot L^{-1}$), and were highest in the Reglone-treated limnocorrals (170 and 3.3 $\mu g \cdot L^{-1}$). Exo-MCYST was correlated with NH_4^+ in the Reglone-treated limnocorrals only (r=0.76; n=17; P<0.001); no correlation between exo-MCYST and NO_3^- was found in any of the limnocorrals or the reference sites.

Pre-treatment K^+ concentrations in the water phase were similar among all limnocorrals and the reference sites (9.0 to 9.2 $mg \cdot L^{-1}$; Fig. 7). Potassium concentrations increased gradually, peaked on day 3 (control, lime-treated limnocorrals, and reference sites) or day 4 (Reglone-treated limnocorral), and remained high until the end of Experiment 2. In ascending order, maximum post-treatment K^+ concentrations were 9.5 (control), 9.7 (reference), 9.9 (lime/alum-treated), and 10.6 $mg \cdot L^{-1}$ (Reglone-treated). The relationship between K^+ and exo-MCYST concentrations in the water phase was significant only in the Reglone-treated limnocorrals (r=0.67; n=17; P<0.004).

Discussion

Over the 7-d experimental period of Experiment 2, increase in phytoplankton volumes at the reference sites coincided with increased P concentrations, possibly due to P release from the lake sediments. Conversely, phytoplankton volumes and P concentrations changed minimally in the control limnocorrals. Despite their differing phytoplankton cell volumes, similar trends in both endo- and exo-MCYST concentrations were noted in both the reference sites and control limnocorrals over the 7-d experimental period. Autoclaved lake sediments did not sorb microcystins from lake water. Although autoclaving could have an effect on sorption characteristics of lake sediments, I considered this unlikely.

Lindholm et al. (1989) also reported that no toxin was detected in the surface sediments of their cyanobacterial-prone lake. Decomposition of released microcystin likely occurs via biotransformation and/or photodecomposition in the water column in lakes. Thus, the persistence of microcystin in the closed-bottomed limnocorrals should be reasonably representative of a natural lake setting.

The decrease in chl*a* concentrations in both lime- and Reglone-treated limnocorrals suggests that both chemicals remove phytoplankton effectively. As lime/alum treatment sediments *Microcystis* cells, post-treatment *Microcystis* volume was lower by an order of magnitude in the lime/alum-treated limnocorrals than in the Reglone-treated limnocorrals for the duration of the experiment. In addition, lime/alum application removed phytoplankton with a concomitant decrease in TDP and TP concentrations, whereas Reglone A did not remove TDP and TP from the water column. As P is the nutrient regulating phytoplankton growth in most lake water (Schindler 1974), lime/alum treatment may have more of a long-term effect than Reglone A on phytoplankton biomass. lime/alum were reported more effective in removing phytoplankton for the poorly buffered stormwater retention lakes in the City of Edmonton (Babin et al. 1992).

Reglone A also removed toxic blooms of *M. aeruginosa* with a concomitant 10-fold increase in exo-MCYST concentrations in the surrounding water compared to pretreatment concentrations. The highest exo-MCYST concentration detected in the lime/alum-treated limnocorrals was 30 times lower than in the Reglone-treated limnocorrals. Similar results were obtained in recent laboratory experiments (Lam et al. in press b; Chapter III). Previous work on lime treatment also recommended maintaining water pH at <10 to avoid pH shock to aquatic life (Murphy and Prepas 1990). In Experiment 1, when water pH increased to almost 11 in the limnocorral, exo-MCYST concentrations increased. Thus, appropriate pH control with lime/alum will minimize the potential health risk where microcystins are present.

In addition, the maximum exo-MCYST concentration in the water phase of the Reglone-treated limnocorral was about 330% higher than that which was initially detected inside the cell. As the commonly used C-18 cartiridge plus acetic acid extraction will not extract hydrophobic endo-MCYST, this suggested some of the toxin concentrations

reported from phytoplankton blooms could have been several times more toxic than that reported when C-18 cartridge plus acetic acid extraction was used. The limitation of the present endo-MCYST extraction however, does not impact on the results from this study, as it was comparative.

In the Reglone-treated limnocorrals, exo-MCYST concentrations increased continuously from days 0 to 2, and remained at high concentrations from days 2 to 7 (3.7 and 3.4 $\mu g \cdot L^{-1}$, respectively); exo-MCYST did not appear to undergo any apparent degradation such as photolysis and biotransformation from days 2 to 7 (*i.e.*, a 5-d period). This result appears to contradict with some laboratory experiments in which MCLR was susceptible to photodecomposition (Tsuji et al. 1994) and exponential biotransformation (Lam et al. in press b).

Photodegradation of exo-MCYST in the presence of sunlight is dependent on the water-extractable cyanobacterial pigment concentrations (reported as freeze-dried weight of cyanobacteria$\cdot L^{-1}$; Tsuji et al. 1994). Results of a 29-d laboratory study (Tsuji et al. 1994) indicated that when the pigment concentrations was ≤ 0.005 $g \cdot L^{-1}$, no photodegradation of exo-MCLR occurred. Only when the pigment concentration reached either 0.1 or 1 $g \cdot L^{-1}$, about 50 and 95% of MCLR was degraded, respectively. In recent laboratory experiments (Lam et al. in press b) with phytoplankton from a lake, concentrated up two order of magnitude, caused a release of water soluble pigments which turned the surrounding water dark green (Lam et al. in press b). In the limnocorral experiment however, lake water in both the Reglone-treated and control limnocorrals had a light yellowish color; the phytoplankton biomass in the present study (0.002 $g \cdot L^{-1}$; dry weight) was about 500 times lower than that used in Lam et al.'s laboratory experiments (1 $g \cdot L^{-1}$). Based on the study by Tsuji et al., photodegradation of exo-MCYST was not expected in the Reglone-treated limnocorrals. Results from the Reglone-treated limnocorrals suggest that photodegradation may play a minor role in the disappearance of exo-MCLR from lake water, given that a chla concentration of 1.5 $mg \cdot L^{-1}$ (equivalent to 0.1 $g \cdot L^{-1}$ water extractable cyanobacterial pigment) is uncommon even in hypereutrophic lakes.

When similar experiments with Reglone A were done in a laboratory with fluorescent light (no photolysis) and 1 $g \cdot L^{-1}$ phytoplankton biomass (dry weight), the half-life of dissolved MCLR ranged from 0.5 to 1.6 d (Lam et al. in press b). The limnocorrals study suggested that exo-MCYST might have a half-life of ≥ 5d. At a half-life of ≥ 5d, it would take at least 3 weeks for 90% or over one month for 99% of the released toxin to be degraded. The biotransformation rate of exo-MCYST was expected to be longer in lakes than in laboratory experiments; the high phytoplankton biomass used in laboratory experiments and other features of the laboratory setting may have resulted in a microbial density which enhanced biotransformation rates over the lake setting.

Exo-MCYST in Lake Centenary, Australia, persisted at high concentrations for 9 d before biotransformation began (Jones and Orr 1994). A similar initial lag phase for biotransformation (7 to 10 d) was also observed in laboratory experiments when purified MCLR was incubated with microbial from sewage effluent (Lam et al. in press a) or lake water (Jones et al. in press). It is therefore possible that the lag-period of biotransformation, instead of half-life of exo-MCYST, was ≥ 5d. Thus, to use biotransformation as a means to remove exo-MCYST in water treatment may present a challenge because of the long adaptation period required.

In addition to exo-MCYST, ions such as P, N, and K^+ will also be released into the water phase as phytoplankton cells decay. However, none of these parameters were correlated with exo-MCYST concentrations at the reference sites, or in the control and lime/alum-treated limnocorrals. Except for NO_3^-, ions such as TDP, TP, NH_4^+ and K^+ were highly ($P < 0.005$) correlated with exo-MCYST concentrations only for the Reglone-treated limnocorrals. The predicability of these parameters ranged from 38 to 65% (for K^+ and NH_4^+, respectively); however, it is not recommended to rely on these parameters for providing clear warnings of the presence of dissolved cyanobacterial toxins after chemical treatment. Until more information on the nature and extent of nutrient release (*i.e.*, K^+) from phytoplankton is known, direct monitoring of microcystin concentrations in drinking water lakes is needed for health risk assessments.

In contrast to some previous laboratory experiments, the observed increase in nutrient concentrations in the limnocorral study only partially agreed with that expected Redfield

Ratio of 42:7:1 (the ratio of C:N:P by weight incorporated in phytoplantkon tissue). Assuming the carbon content of freshwater phytoplankton is about 50% of dry-weight (uncorrected for ash; Reynolds 1984), a phytoplankton biomass of about 2 $mg \cdot L^{-1}$ at day 0 would be equivalent to 1 mg $carbon \cdot L^{-1}$. When phytoplankton decayed and dissociated in the Reglone-treated jars after chemical addition, based on the Redfield ratio, the concentrations of N and P in the Reglone-treated jars would increase by approximately 167 and 24 $\mu g \cdot L^{-1}$, respectively. However in the Reglone-treated limnocorrals, average NH_4^+ and TP concentrations increased by 150 and 115 $\mu g \cdot L^{-1}$, respectively, over the 7-d period. Thus, NH_4^+ increase in the Reglone-treated limnocorrals was likely from dissociated phytoplankton. The greater than predicted increase in P in the Reglone-treated limnocorrals remains to be resolved.

In short, the field experiments support the concept that chemicals that precipitate or sediment cyanobacteria cells (such as lime and alum) appear to be 'safer' than chemicals that cause cell destruction (such as Reglone A and chlorine) for the control of microcystin-containing phytoplankton blooms (Lam et al. in press b). Lime/alum removes MCYST-containing phytoplankton effectively with a concomitant decrease in both TDP and TP concentrations. There was no increase in exo-MCYST concentrations in the surrounding water when compared with the control. Reglone A treatment removed phytoplankton effectively but resulted in increased TDP, TP, and exo-MCYST concentrations in the surrounding water; exo-MCYST did not degrade for at least 5 d. In addition, laboratory experiments indicate that lake sediments did not sorb microcystin from lake water. The persistence of exo-MCYST concentrations after Reglone A addition is likely to increase chronic health risks in drinking water. Thus a suitable withholding period (>> 7d), together with exo-MCYST monitoring is required after Reglone A addition, or any other chemical that destroys cells, before the water can be used as a drinking water supply or for contact recreation.

Table 1. Percent composition of freeze-dried lake sediments collected from Little Beaver Lake on 27 May 1992 based on the Wentworth size class distribution with the SediGraph method.

Sediment composition		percentage
Clay	(dia. <3.9 μm)	52
Fine to medium silt	(3.9 < dia. <31 μm)	44
Coarse silt	(31 < dia. <62 μm)	3.5
Very fine sand	(62 < dia. <125 μm)	0.5

Table 2. Summary of the characteristics of the control, lime/alum-, and Reglone-treated limnocorrals and the reference sites in Experiment 2 before chemical additions: Chlorophyll *a* concentrations, phytoplankton volume, species composition, and endo- and exo-microcystin (MCYST) concentrations (μg microcystin-LR equivalent·L^{-1}). Data are means ± standard error.

	Control	Lime/Alum	Reglone A	Reference
Chlorophyll *a* (μg·L^{-1})	65 ± 19	69 ± 15	91 ± 19	33 ± 1.0
Phytoplankton ($mm^3 \cdot L^{-1}$) composed of	42 ± 6.1	42 ± 6.4	45 ± 4.0	13 ± 1.7
M. aeruginosa (%)	37	26	22	64
Aph. flos-aquae (%)	62	73	77	32
Endo-MCYST (μg·L^{-1})	0.66 ± 0.24	0.73 ± 0.41	1.09 ± 0.33	1.52 ± 0.20
Exo-MCYST (μg·L^{-1})	0.29 ± 0.06	0.33 ± 0.00	0.32 ± 0.01	0.25 ± 0.06

Table 3. Volume of *Aph. flos-aquae* and *M. aeruginosa* ($mm^3 \cdot L^{-1}$) in the control, lime/alum-, and Reglone-treated limnocorrals, and at the reference sites over the 7-day period in Experiment 2. Data are means ± standard error.

Species	Day	Control	Lime/Alum	Reglone A	Reference
Aph. flos-aquae	0	26 ± 0.4	31 ± 5.4	35 ± 3.3	4.2 ± 0.4
	1	26 ± 8.3	1.9 ± 0.1	12 ± 1.2	2.7 ± 1.2
	2	33 ± 14	1.6 ± 0.9	7.1 ± 2.1	2.7 ± 1.4
	3	27 ± 11	2.8 ±1.4	6.0 ± 0.6	15 ± 2.5
	4	33 ± 13	4.5 ±1.8	13 ± 2.1	9.2 ± 1.8
	7	32 ± 17	5.1 ± 2.7	3.6 ± 0.5	30 ± 4.9
M. aeruginosa	0	16 ± 1.5	11 ± 1.1	9.8 ± 1.9	8.5 ± 1.5
	1	5.0 ± 2.9	0.2 ± 0.1	6.6 ± 1.3	7.3 ± 2.5
	2	6.2 ± 2.1	1.7 ± 1.7	10 ± 3.2	6.3 ± 4.0
	3	2.9 ± 1.7	0.1 ± 0.0	3.8 ± 1.0	2.5 ± 0.2
	4	2.8 ± 0.6	0.1 ±0.0	5.4 ± 1.0	5.2 ± 1.7
	7	1.7 ± 1.4	0.6 ± 0.4	5.1 ± 0.6	7.2 ± 2.2

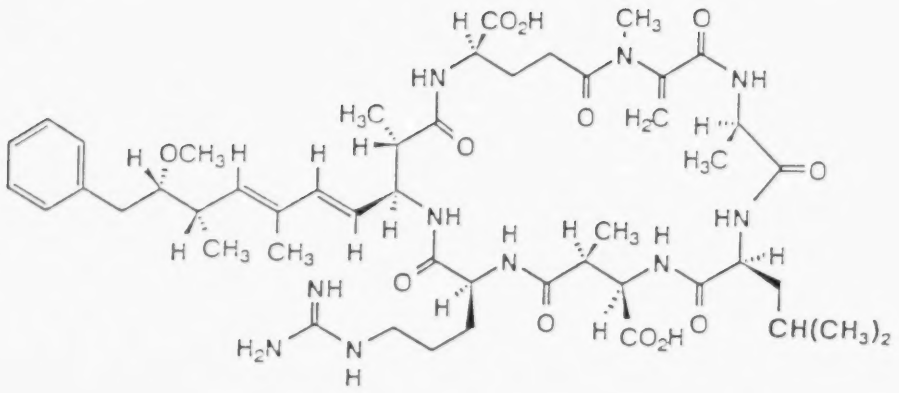

Fig. 1. Structure of microcystin-LR.

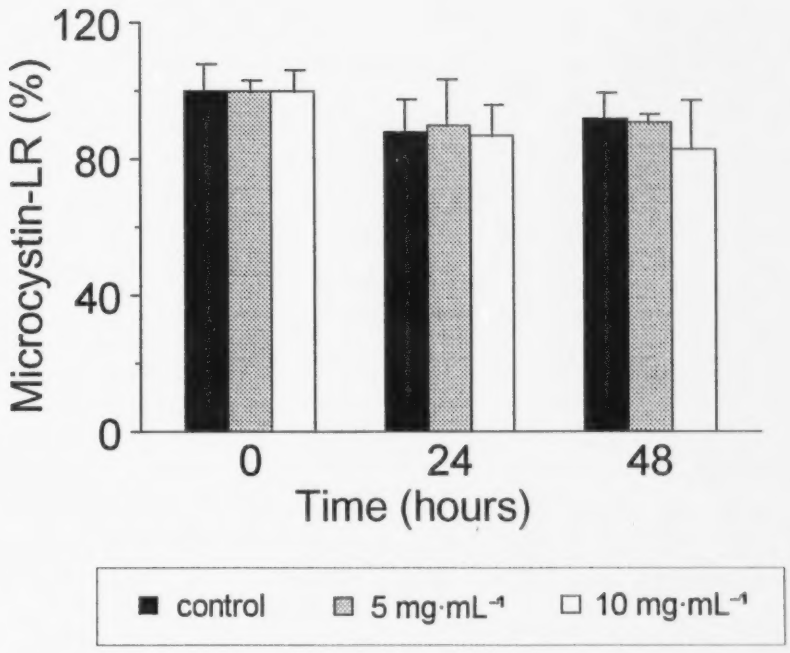

Fig. 2. MCLR concentration at the beginning of the experiment (0 hour) was 100 $\mu g \cdot L^{-1}$. MCLR concentration in the double distilled water after exposure to lake sediment is expressed as a percent of initial MCLR concentration. Two sediment concentrations, 5 and 10 $g \cdot L^{-1}$ were used in this experiment. Values shown are mean ± 1 standard error (*SE*).

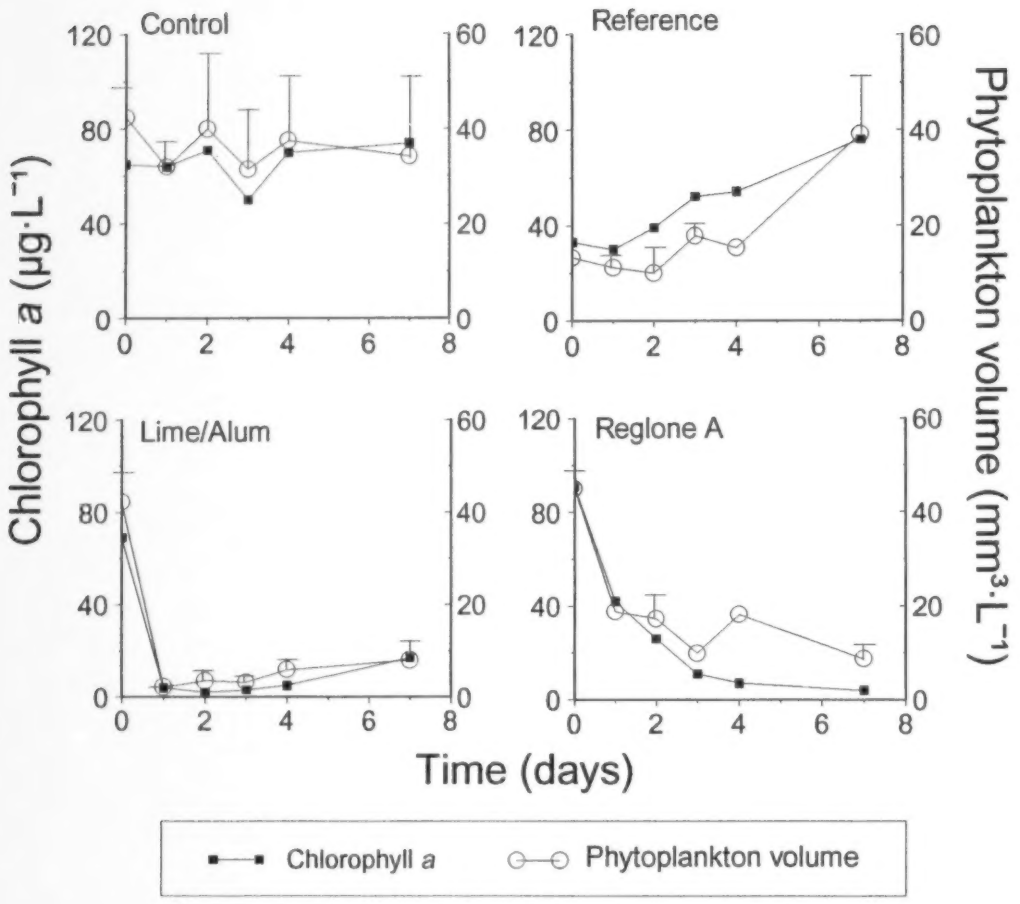

Fig. 3. Average chlorophyll *a* concentrations and phytoplankton volumes in all limnocorrals and reference site over the 7-d period and just prior to chemical addition (day 0) in Experiment 2. Bars indicate 1 *SE* of phytoplankton volume. For clarity, *SE* of chlorophyll *a* are not shown.

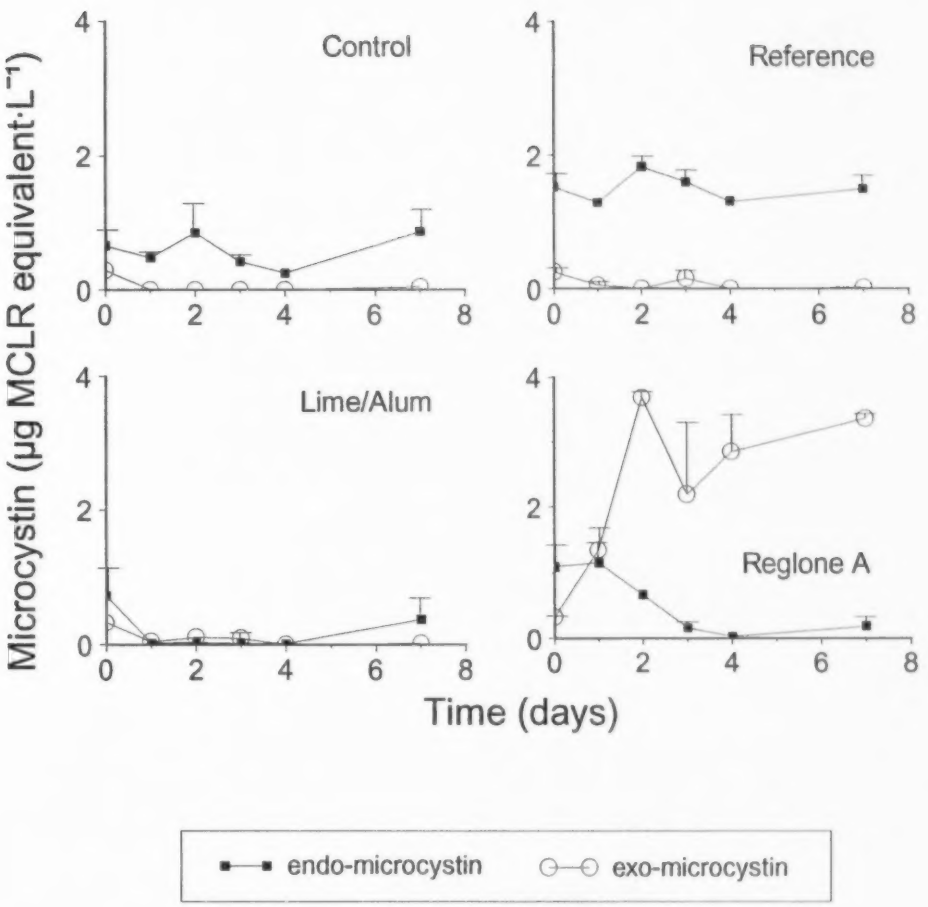

Fig. 4. Average endo- and exo-microcystin concentrations in all limnocorrals and reference site over the 7-d period and just prior to chemical addition (day 0) in Experiment 2. Bars indicate 1 *SE*.

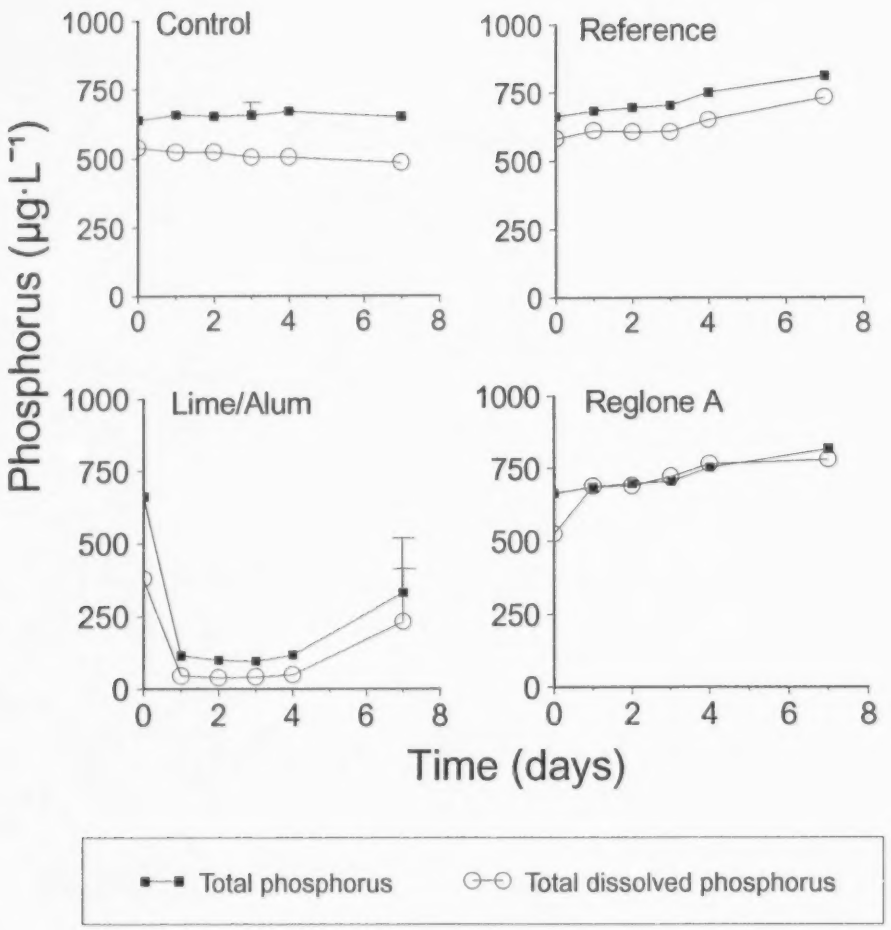

Fig. 5. Average total and total dissolved phosphorus concentrations in all limnocorrals and reference site over the 7-d period and just prior to chemical addition (day 0) in Experiment 2. Bars indicate 1 *SE*. For clarity, *SE* ≤5 % of the mean concentration is not shown.

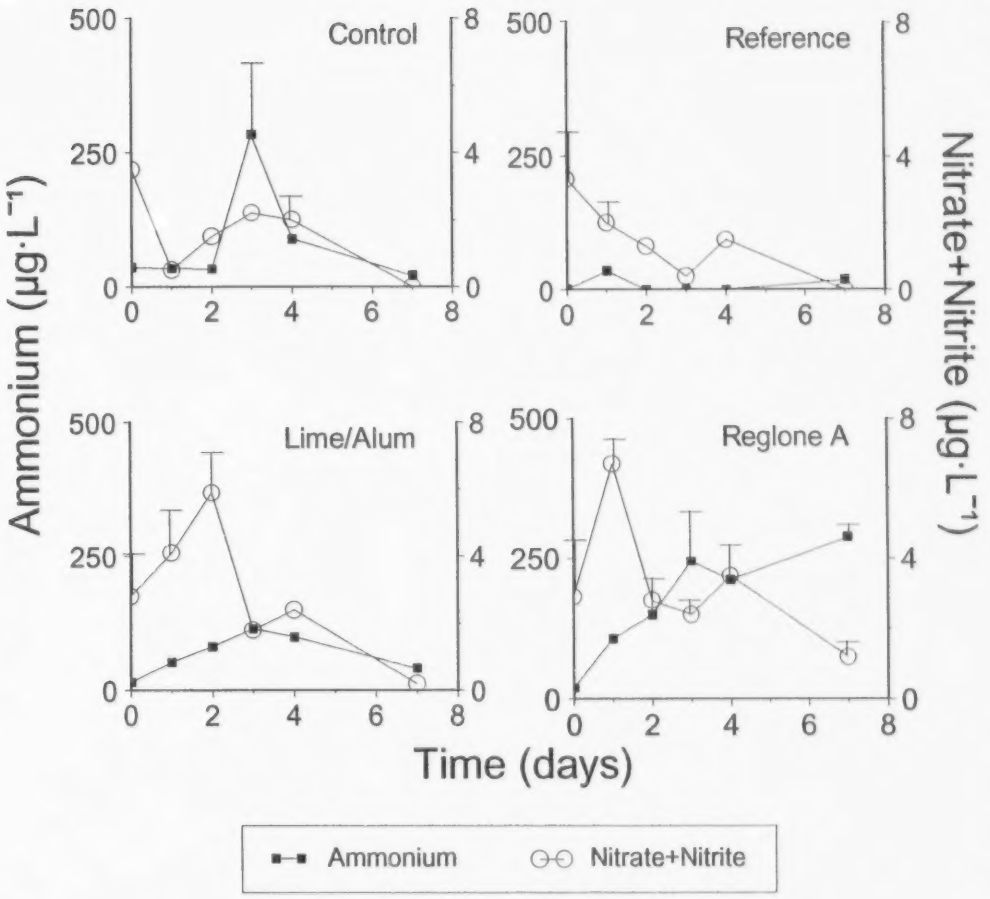

Fig. 6. Average ammonium and nitrate+nitrite concentrations in all limnocorrals and reference site over the 7-d period and just prior to chemical addition (day 0) in Experiment 2. Bars indicate 1 *SE*.

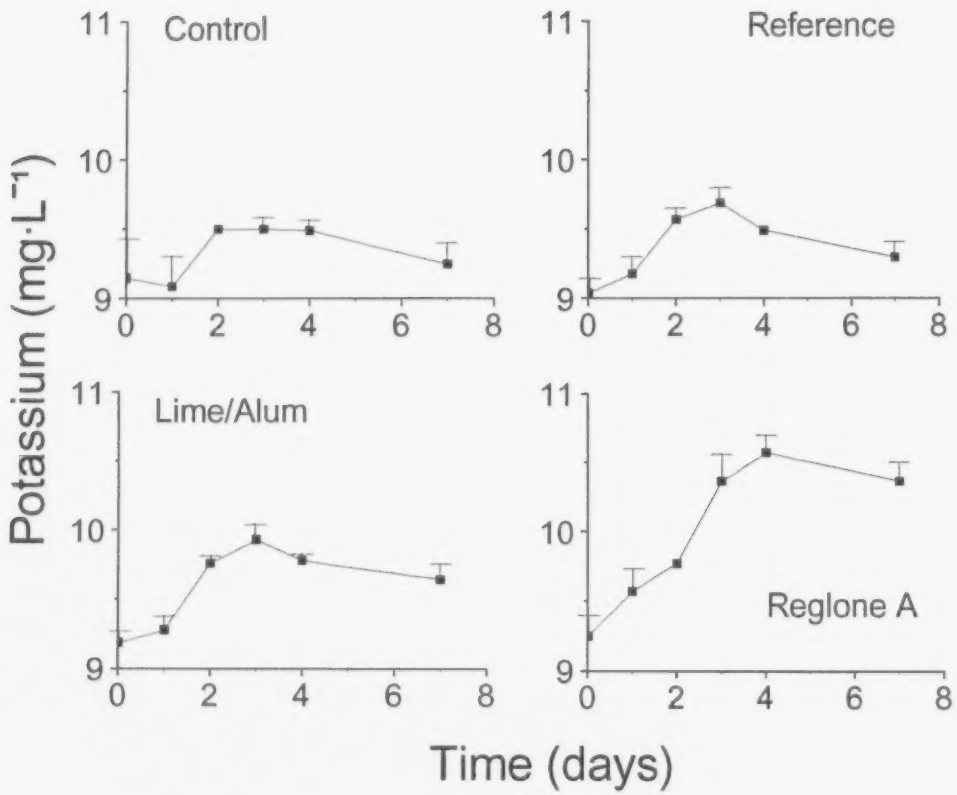

Fig. 7. Average potassium concentrations in all limnocorrals and reference site over the 7-d period and just prior to chemical addition (day 0) in Experiment 2. Bars indicate 1 *SE*.

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V. Biotransformation of the cyanobacterial hepatotoxin microcystin-LR, as determined by HPLC and protein phosphatase bioassay[5]

Introduction

Microcystins are a group of intracellular toxins that act on the liver and are therefore referred to as hepatotoxins. They are commonly associated with freshwater cyanobacterial blooms. Originally, the structure of microcystin, a cyclic peptide, was found to consist of five 'invariable' amino acids (D-alanine, erythro-β-methylaspartic (MAsp), D-glutamic acid (Glu), N-methyldehydroalanine (Mdha), and 3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl-deca-4,6-dienoic acid (Adda)), and two variable L-amino acids (Gathercole and Thiel 1987; Carmichael 1988; Harada et al. 1988). It was soon found however that the structure of the 'invariable' amino acids also differed regarding the demethylation of the amino acids Mdha and/or MAsp (Carmichael 1988), and modification in the double bonds of the Adda moiety (Namikoshi et al. 1991; Namikoshi et al. 1992; Sivoven et al. 1992). Nevertheless, the Adda moiety is an amino acid unique to the cyanobacterial hepatotoxins found to date. Microcystin-LR (MCLR) (Fig.1) is the most common hepatotoxin found in cyanobacterial blooms in Alberta, Canada (Kotak et al. 1993). The two suffixes L and R represent the variable amino acids leucine and arginine, respectively.

The occurrence of toxic cyanobacterial blooms in lakes has resulted in animal poisonings world-wide and raised public health concerns because such lakes are often used as drinking water supplies (Skulberg 1984; Meyer 1987; Carmichael and Falconer 1993). Microcystins are typically bound within cyanobacterial cells and they are released into the surrounding water when there is a change in cell permeability due to: cell aging (Berg et al. 1987), photo-oxidation (Eloff et al. 1976), bacterial-lysis (Shilo 1971; Stewart and

5. A version of this chapter has been accepted for publication. A. K.-Y. Lam, P. M. Fedorak, and E. E. Prepas. *Environ. Sci. Technol.*

Draft 1977), or chemical treatment (Bourke et al. 1983; Kenefick et al. 1993; Lam et al. in press). Microcystins may also be released into the gastrointestinal tract of an organism when cyanobacterial cells are consumed. Microcystins are resistant to common proteolytic hydrolysis and may therefore be resistant to degradation in tissues (Falconer et al. 1986). Some microcystins, such as MCLR (LD_{50} of 0.05 $mg \cdot kg^{-1}$ in mice by intraperitoneal injection; Carmichael 1988) are more acutely toxic than sodium cyanide (LD_{50} of 4.3 $mg \cdot kg^{-1}$ in mice by intraperitoneal injection) (Sax and Lewis 1989). MCLR also inhibits protein phosphatase-1 (PP-1c; IC_{50} of 0.1 nM) at a much lower concentration than the diarrehetic shellfish toxin, okadaic acid (IC_{50} of 10 nM) (MacKintosh et al. 1990). Laboratory and in situ studies have shown that MCLR can potentially accumulate in freshwater clams such as *Anadonta cyanea* (Eriksson et al. 1989; Lindholm et al. 1989) and *Mytilus edulis* (Falconer et al. 1992) and may have an impact on fish health (Shelubsky 1951; Philips et al. 1985; Rabergh 1991; Rodger 1994).

There is limited information on the persistence of MCLR in water environments. Findings on the modification of MCLR by microbial activity are rare and none have assessed the changes in the structure of MCLR and its PP-1c inhibition ability after incubation with aerobic microbes. For example, one laboratory experiment could not detect biodegradation of a microcystin toxin (Kiviranta et al. 1991), whereas others have suggested that the loss of MCLR in the water was due to microbial activity (Bourke et al. 1983; Kenefick et al. 1993; Lam et al. in press). MCLR is not completely removed by conventional water treatment processes such as flocculation-filtration-chlorination (Keijola et al. 1988; Himberg et al. 1989) unless expensive activated carbon filtration is utilized (Keijola et al. 1988; Himberg et al. 1989; Lambert 1993). In addition, Jones et al. (in press) have recently demonstrated degradation of MCLR by aquatic bacteria and found that 10 to 50 $\mu g \cdot L^{-1}$ MCLR remained in solution at the end of their 14-d experiments. These findings imply that released MCLR remain at detectable concentrations in the water for a substantial period. It is important to predict the residence time of MCLR in a water body for public health protection. One way to achieve this objective is to understand the degradation process(es) of MCLR.

The objective of this study was to determine whether the concentration of purified MCLR would decrease (analysed with high performance liquid chromatography; HPLC) during incubation under aerobic conditions with a diverse microbial community, orginating from sewage effluent from a domestic activated sludge process. Possible modification in the structure of MCLR and its PP-1c inhibition ability after biotransformation were also assessed.

Materials and Methods

Biotransformation. Final effluent from the activated sludge wastewater treatment plant in Edmonton, Alberta was used as a source of heterotrophic microbes. This effluent typically has 13 $mg \cdot L^{-1}$ total suspended solids and 9 $mg \cdot L^{-1}$ carbonaceous biochemical oxygen demand (Mitchell C. personal communication). Autoclaved effluent with the addition of 40% formalin (100 µL per 25 mL sample) was used as a sterile control. Purified MCLR from *Microcystis aeruginosa* was supplied by Dr. Wayne Carmichael (Wright State University, Dayton, Ohio). The MCLR concentrations used in these experiments ranged from 210 to 1620 $\mu g \cdot L^{-1}$, which are higher than the concentrations of a few micrograms per liter observed in lake water (Watanabe et al. 1992). In this paper, the detection limits of MCLR by HPLC and protein phosphatase (PP) bioassay were 40 and 0.2 $\mu g \cdot L^{-1}$, respectively. High experimental concentrations were necessary to ensure detection of changes in MCLR concentrations. All cultures were incubated on a shaker at 120 rpm and in the dark at 25°C.

Three separate experiments were conducted. The extent of MCLR modification by microbial activity and the variation among replicates were investigated in Experiment 1. Each replicate contained 250 mL of the sewage effluent in a 500-mL Erylenmeyer flask which was immediately spiked with purified MCLR to a concentration of 210 $\mu g \cdot L^{-1}$. Triplicate samples of sewage effluent with no MCLR addition were used as a reference to monitor bacterial numbers. Triplicate sterile controls were prepared in the same manner. Samples (25 mL) were collected from each flask at 0, 3, 7, 14, 21, and 28 d, and concentrated with a disposable Sep-Pak C-18 cartridge (Supelco) for MCLR analysis by

HPLC at 238 nm. Details of sample cleanup and analysis are described in Kenefick et al. (1993). Standard bacterial plate counts for the control, experimental, and reference cultures were also performed with Plate Count Agar (Difco; Detroit, Mich.) in triplicate at all sampling times. Inoculated agar plates were incubated at 25°C for 3 d. Bacterial densities were expressed as colony forming units per milliliter of effluent ($CFU \cdot mL^{-1}$).

Experiment 2 examined the changes in MCLR-induced PP-1c inhibition ability, and determined the reproducibility of Experiment 1. Two live cultures and one sterile control were used. For each of the duplicate cultures, 500 mL of sewage effluent was placed in a 1-L Erylenmeyer flask. The sterile control contained 500 mL of autoclaved sewage effluent with the addition of 40% formalin (100 µL per 25 mL). At the beginning of the incubation, each flask received MCLR to a final concentration of 580 $\mu g \cdot L^{-1}$. Samples (25 mL) were taken every 2 to 4 d to monitor for MCLR. The samples were first passed through a Sep-Pak C-18 cartridge to concentrate the toxin (Kenefick et al. 1993) and the concentrate was divided into two portions: one for HPLC analysis and one for the PP bioassay. By day 16, the MCLR concentrations in the duplicate cultures were barely detectable; therefore one flask ('flask A') was spiked with MCLR to give a concentration of 1600 $\mu g \cdot L^{-1}$, and the other ('flask B') was spiked to give a concentration of 720 $\mu g \cdot L^{-1}$. Similarly, the sterile control was spiked to give a concentration of 680 $\mu g \cdot L^{-1}$. Samples (25 mL) were removed twice per day between days 16 and 20, and were analysed by HPLC for MCLR and PP bioassay for total microcystins. Results of the PP bioassay were reported as MCLR equivalent.

Experiment 3 was performed as a preliminary assessment of the structural changes in MCLR after incubation with microbes. One live culture and one sterile control were used. HPLC analyses were done at the beginning and end (day 15) of the experiment, the column effluent was monitored at wavelengths of 238 and 210 nm. The former wavelength detects the aromatic portion of the Adda side-chain (Scott 1964), and the latter detects the peptide bonds in the ring of MCLR (Boland et al. 1993).

Protein Phosphatase Bioassay. Phosphatase assay is commonly used as a measure of toxicity because reversible protein phosphorylation by protein kinases/phosphatase is a universal regulatory device in eukaryotes for modulating the activity of intracellular proteins in response to extracellular signals (Holmes and Boland 1993). In this assay, phosphorylase a was radiolabelled with ^{32}P at the beginning of the experiments. The dephosphorylation of phosphorylase a to phosphorylase b was catalyzed by PP-1c. MCLR specifically inhibits PP-1c; thus, the concentration of cleaved ^{32}P is inversely proportional to the concentration of MCLR present in the sample. The same PP bioassay has been used for okadaic acid (Holmes 1991).

Statistical Analysis. Repeated measures on Analysis of Variance ($P < 0.05$) was used to evaluate the significance of these experimental results (Wilkinson et al. 1992).

Results and Discussion

When purified MCLR was exposed to a microbial community from the Edmonton wastewater treatment plant, significant decreases in MCLR concentration were detected in all experiments. The concentration of MCLR in the sterile control flasks remained relatively constant over the test periods. In Experiment 1, the concentrations of MCLR in the live cultures and sterile controls were the same on day 7 (Fig. 2). MCLR concentrations had decreased to 40% of the initial concentration in the culture on the following sampling date (day 14). MCLR continued to decrease in the test culture until day 27 when it was not detectable (Fig. 2).

There was no significant difference between the bacterial densities in the test and reference cultures (without MCLR) over the 27-day experimental period in Experiment 1. The bacterial count in the test culture increased from $(3.7 \pm 0.4) \times 10^4$ CFU·mL^{-1} at day 0 to $(1.5 \pm 0.5) \times 10^5$ CFU·mL^{-1} at day 7, followed by a gradual decrease to $(2.6 \pm 0.6) \times 10^4$ CFU·mL^{-1} at day 21. The initial increase in CFU·mL^{-1} in the test culture suggested that MCLR did not inhibit the growth of the bacterial community in the culture. The decrease in MCLR concentration was not accompanied by an increase in CFU. The lag time (slightly > 7 d; Fig. 2) suggests that the microbes required an adaptation period prior

to transforming the MCLR. Similar lag times of 4 to 12 d prior to MCLR degradation was reported when bacteria from lake water were used (Jones et al. in press).

When sampling was intensified between day 7 and 14 in Experiment 2, there was a decline in MCLR concentration after an initial 10-day lag period (Fig. 3). When MCLR was added again at day 16, its concentration immediately decreased in flask A. In flask B, however, there appeared to be a short lag period of <1 d before a rapid decrease in MCLR concentration was detected. The concentration of MCLR in the sterile control did not change detectably throughout the 20-d experiment. These results indicated that the bacteria at day 16 had adapted to readily transform MCLR. The concentration of MCLR decreased to $< 0.2\ \mu g \cdot L^{-1}$ within 0.8 and 1.9 d in flasks A and B, respectively (Fig. 3). The higher transformation rate of MCLR in flask A than in flask B might be due to a higher bacterial density in flask A than in flask B ($8.4 \pm 0.7 \times 10^5$ and $0.9 \pm 0 \times 10^5$ $CFU \cdot mL^{-1}$, respectively). The dependency of biotransformation rate on bacterial density is a common phenomenon.

Results of the PP bioassay (Fig. 4) for MCLR were similar to those from the HPLC analysis. MCLR lost its PP-1c inhibition ability after incubation with microbes. It therefore appears that biotransformation can be used to reduce the tumor promoting ability of MCLR. There are over 50 different microcystins identified to date, and standard solutions are often not available for these microcystins using HPLC analysis. PP bioassay measures the total microcystin concentrations as nanograms of MCLR equivalent per liter of water, and does not require all microcystin standard solutions. Therefore, PP bioassay appears to be a quick monitoring tool for total microcystin concentration in drinking water.

Changes in the structure of MCLR were assessed in Experiment 3. At the beginning of the experiment, the MCLR peak was detected at both wavelengths monitored (210 and 238 nm) from samples of the sterile control and live culture. At day 15, the MCLR peak in the sterile control was still detected at both wavelengths (Fig. 5a, b). In the live culture however, there was a 160- and 12-fold decrease in peak areas detected at 238 and 210 nm, respectively, in the day 15 samples (Fig. 5c, d). These results indicated that the chromatographic properties of the MCLR had changed and suggested that (a) the 'Adda'

group was modified, resulting in a loss of absorbance at 238 nm (Scott 1964), and (b) the heptapeptide ring of MCLR has been opened or destroyed, resulting in a loss of absorbance at 210 nm (Boland 1993). Thus, MCLR appears to have been extensively degraded after incubation with microbes.

In other studies, loss of toxicity from microcystins has been shown to result from minor changes in Adda moiety, which plays an important role in the toxicity of these hepatotoxins (Namikoshi et al. 1992). For example, hydrogenation or ozonolysis of the diene system in the Adda unit (Dahlem et al. in press) or stereoisomerization of the double bonds in the Adda moiety (Harada et al. 1990) greatly reduces the toxicity of these modified microcystins.

A straight line plot of $\ln(Ct \cdot Co^{-1})$ versus t for the data from Experiment 1 (Fig. 6) indicated that MCLR biotransformation followed a first-order decay as was observed by others (Kenefick et al. 1993; Jones et al. in press). Co represents the MCLR concentration at day 7 and Ct represents the MCLR concentration at time t, thereafter. The first-order rate constant was 0.19 d^{-1} or 4.6 h^{-1}. Using various volumes of a cell-free extract from a MCLR-degrading bacterial culture, Jones et al. (in press) showed first-order rates of MCLR breakdown were between 0.4×10^4 sec^{-1} (1.1 h^{-1}) and 2.8×10^4 sec^{-1} (7.8 h^{-1}). The rate of MCLR decay in our experiment falls within this range.

The half-life of MCLR in Experiment 1 was 3.6 d after day 7 (n=12). Based on the limited data available after the second spiking in Experiment 2, the half-life of MCLR was estimated to be 0.2 and 0.6 d (n=3) in flasks A and B, respectively. The difference in half-life estimates in the two experiments was probably due to the 9-fold higher CFU present in the culture with the fastest rate of MCLR decay in flask A.

Results of the present study also suggest that microbes in a wastewater effluent can be induced to transform MCLR; high microbe densities appear to play a key role in determining the MCLR transformation rate. Kiviranta et al. (1991) examined the production and biodegradation of the hepatotoxin desmethyl-3-microcystin-RR (MCRR) from *Oscillatoria agardhii*. They used 1 mL of cyanobacterial-infested river water per 100 mL of the MCRR-containing culture as their source of microbes. Increased MCRR concentrations in water coincided with decreased MCRR concentrations in cells for both

the axenic and non-axenic cultures. Kiviranta et al. (1991) concluded that biodegradation of the hepatotoxin did not occur, which explained the high concentrations of hepatotoxin present in the water phase at the end of their 8-week study. Jones et al. (in press) showed that degradation of MCLR was observed in 4 of 5 surface water samples in their 14-d experiment.

In contrast, biotransformation of MCLR was detected in all of our experiments. MCLR concentrations were below detection (< 0.2 $\mu g \cdot L^{-1}$ based on PP bioassay) by the end of the experimental period. Purified MCLR was used instead of cyanobacterial cultures to prevent complications due to continuous release of hepatotoxins from cells into the water phase. Also, we used 250 and 500 mL samples of sewage effluent which is known to have a diverse microbial community. The microbial community in the wastewater effluent used in our experiments was more dense than that typically found in lakewater. In addition, the shake-flask method of incubation of our cultures would likely provide much higher dissolved oxygen concentrations than would exist in a lake after the collapse of a cyanobacterial bloom. Thus, biotransformation rates of MCLR by aerobic microorganisms in natural aquatic environments are expected to be slower than the rates measured in our experiment. However, microbial populations in lake water as high as 10^5 $CFU \cdot mL^{-1}$ (by standard plate count method) have been measured after cyanobacteria were chemically treated in the laboratory (Lam et al, in press). Under such circumstances, biotransformation might become the dominant degradation route of MCLR. The half-life of MCLR estimated under such conditions ranged from 0.5 to 2.8 d (Kenefick et al. 1993; Lam et al. in press) which is similar to the half-life of MCLR estimated in our experiments (0.2 to 3.6 d). Thus in lakes, biotransformation may be an important degradation route of MCLR after massive cyanobacterial blooms collapse at the end of the growth season.

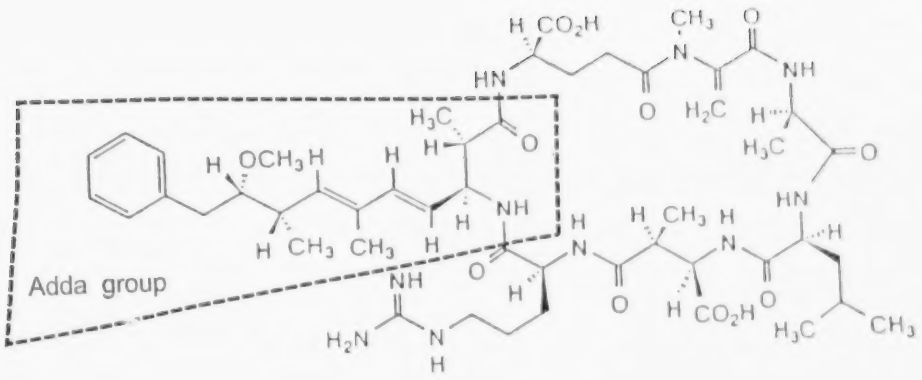

Fig. 1. Structure of microcystin-LR

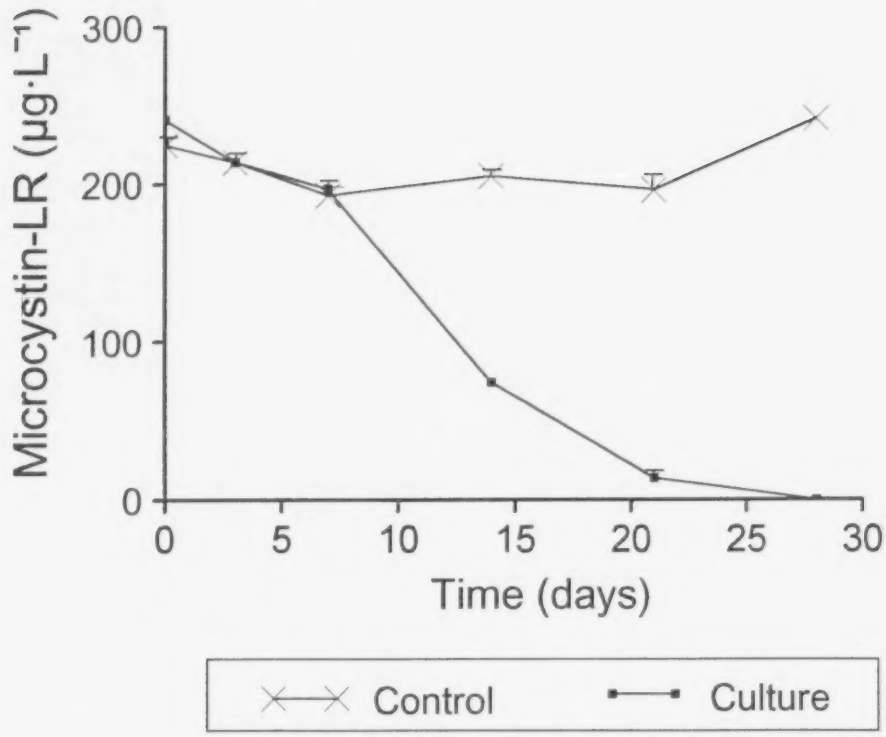

Fig. 2. Average MCLR concentrations in triplicate cultures used in the Experiment 1 with sewage effluent as a source of microbial flora. Most standard error bars are smaller than the symbol.

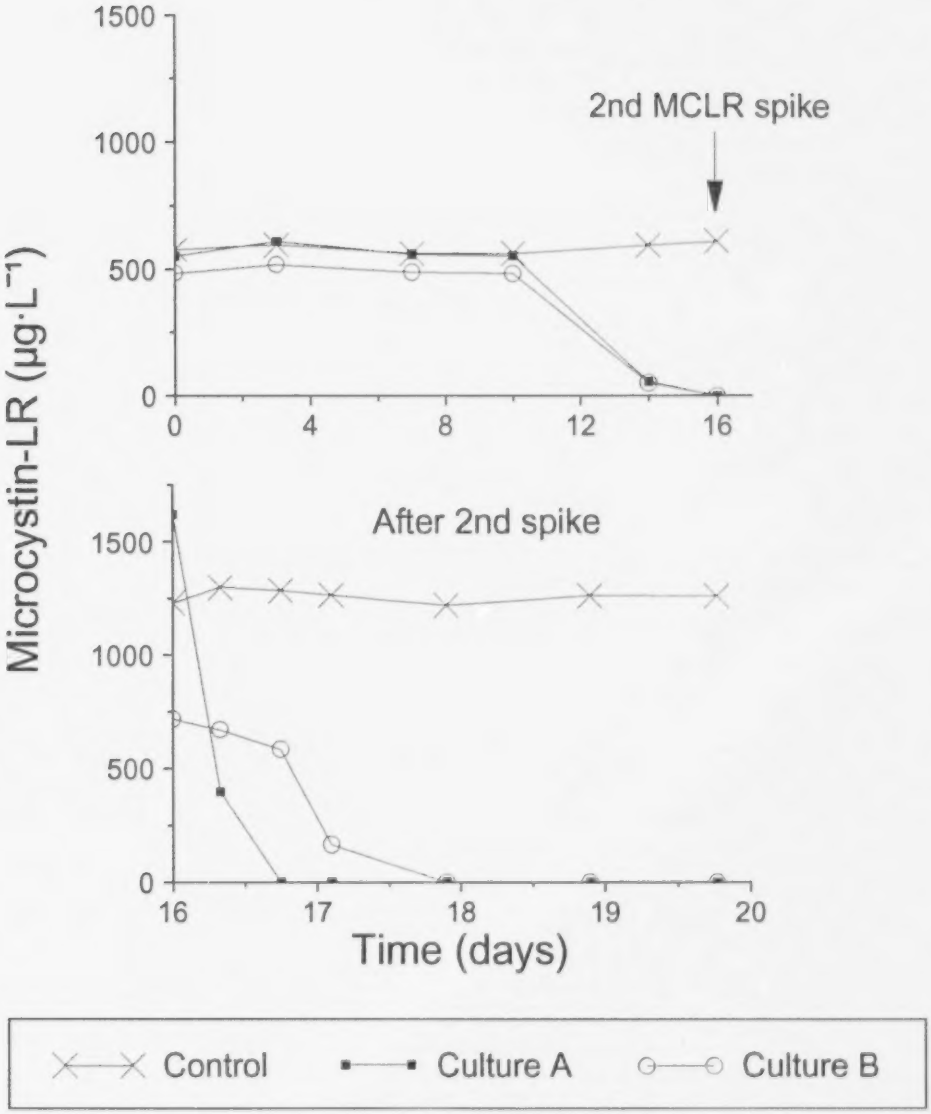

Fig. 3. MCLR concentrations in cultures used in the Experiment 2 with sewage effluent as a source of microbial flora. HPLC used for MCLR analysis.

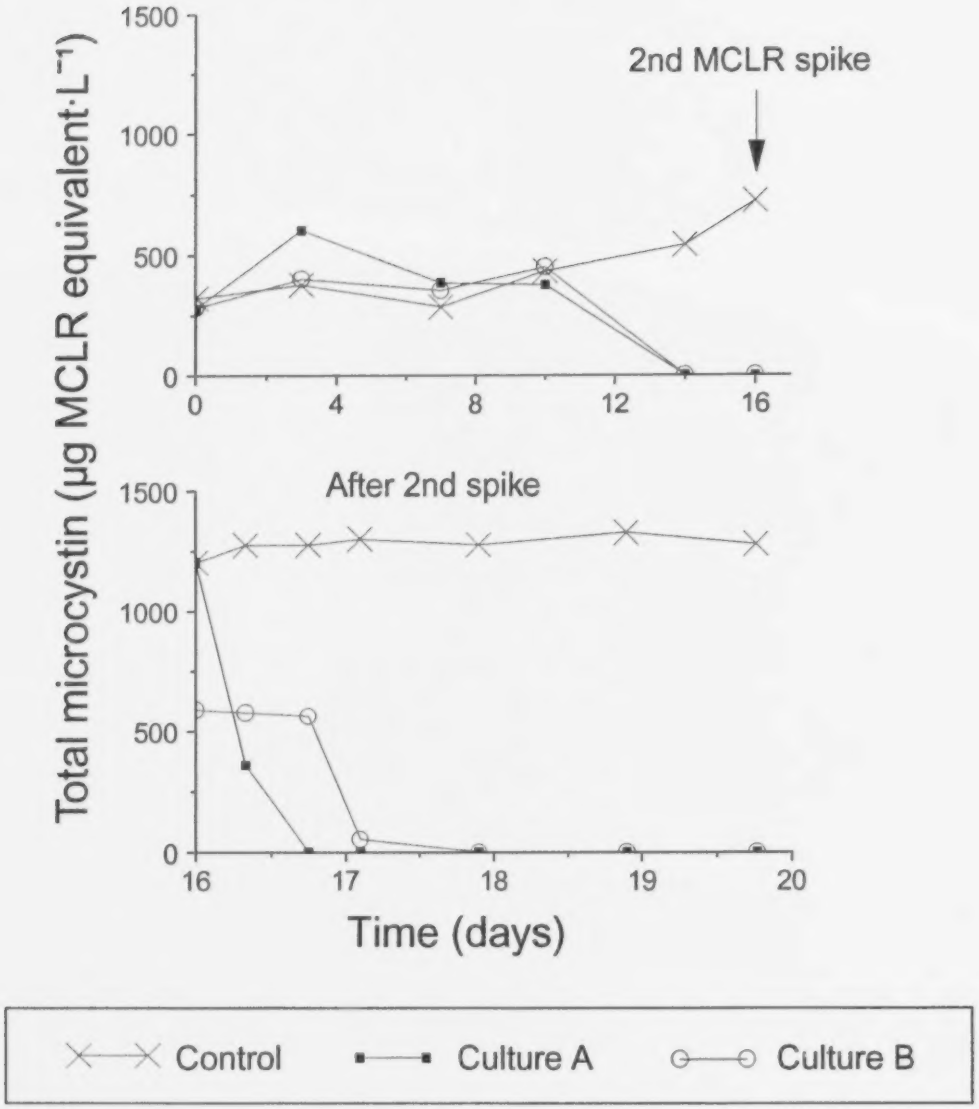

Fig. 4. MCLR concentrations in cultures used in the Experiment 2 with sewage effluent as a source of microbial flora. Protein phosphatase bioassay used for MCLR analysis.

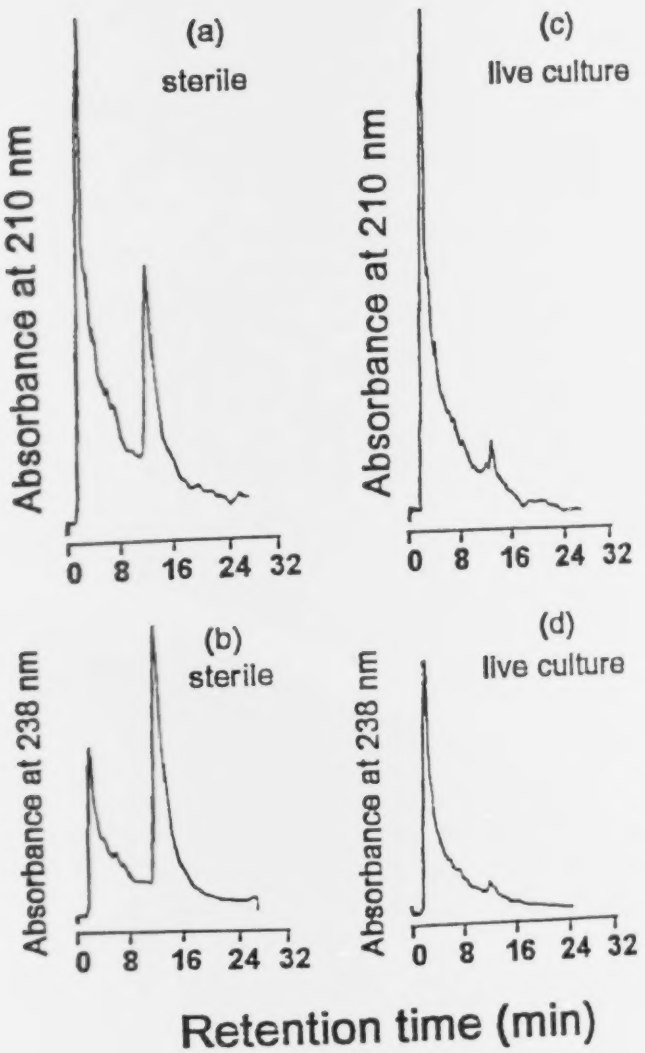

Fig. 4. HPLC chromatogram for Experiment 3 showing MCLR peak at 11.5 min for both the sterile control (a, b) and the live culture (c, d) on day 15. Duplicate analysis of each sample were done. For one analysis, the column effluent was monitored at 210 nm (a, c) and for the other analysis, it was monitored at 238 nm (b, d).

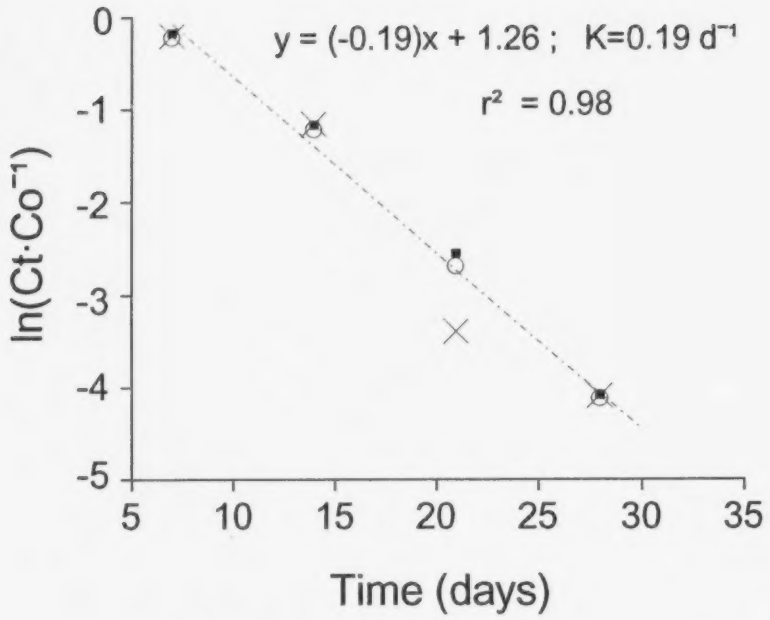

Fig. 6. Half-life estimation of MCLR exposed to a microbial population in the sewage effluent. Data are from Experiment 1 (Fig. 2). Results from each of the cultures are indicated by different symbols.

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VI. General discussion and conclusions

(1) *Occurrence of the cyanobacterial toxin, Microcystin-LR (MCLR)*

Freshwater cyanobacteria are known to produce a family of at least 50 cyclic hepatotoxins called microcystin (MCYST). Among the microcystin analogues, MCLR was the major MCYST found in the three drinking water supply lakes (Little Beaver, Coal, and Driedmeat) in Alberta in 1990 (Kenefick et al. 1992; Kotak et al. 1993; Craig et al. 1993). Over the three study years (1991-1993), MCLR concentrations ranged from ≤ 1 $\mu g \cdot g^{-1}$ to 1300 $\mu g \cdot g^{-1}$ biomass (by high performance liquid chromatography). The MCLR concentrations were variable within each lake and among years, reflecting the variability of both the volume of *Microcystis* and the production of MCLR by *Microcystis* cells within lakes and over time.

When all data from the 3-year study of these lakes were pooled, over 40% of the variability in MCLR could be explained by the abundance of *Microcystis*. *Microcystis* was the dominant genera in phytoplankton blooms (< 50% of phytoplankton volume) over a wide range of water temperatures (12-20°C). The abundance of *Microcystis* was strongly positively correlated with total phosphorus (TP) concentration and pH (r=0.54 and 0.52, respectively, n=137, P<0.0001) and negatively correlated with TN:TP ratio and Secchi depth (r= -0.71 and -0.50, n=39 and 127, respectively, P<0.0001). As MCLR concentration in these lakes was produced by *Microcystis*, MCLR was also positively correlated to TP concentration and pH, and was not correlated with water temperature (Kotak et al. submitted). When detectable MCLR ($\mu g \cdot g^{-1}$) was expressed as per *Microcystis* cells, the intracellular MCLR concentration apparently consisted of three phases over the lifetime of a bloom: Phase 1, decline in intracellular MCLR; Phase 2, stable intracellular MCLR; Phase 3, increase in intracellular MCLR. Thus, seasonal changes in *Microcystis* and intracellular MCLR concentrations were variable both among lakes and years; however, the notion that blooms may be toxic one day and not the next was not supported in this study.

(2) *Chemical treatment options for removing hepatotoxic cyanobacterial blooms*

Use of chemicals to control phytoplankton blooms containing intracellular MCYST could increase the potential health risks in drinking water supplies if the phytoplankton release intracellular toxins. The effects of two alternative approaches to chemical removal of phytoplankton was examined first in a laboratory setting with freshly collected phytoplankton (1 $g \cdot L^{-1}$ phytoplankton biomass at 182 to 837 μg MCLR$\cdot g^{-1}$ biomass; dry weight) and then in the lake setting (2 $mg \cdot L^{-1}$ phytoplankton biomass at 0.48 to 1.4 μg MCYST$\cdot g^{-1}$ biomass) with closed-bottom limnocorrals. Six chemical treatments were categorized into two groups; Group 1 chemicals disrupt cell functions and induce cell lysis; they include Reglone A, chlorination (NaOCl), potassium permanganate, and Simazine; and Group 2 chemicals precipitate (or coagulate) phytoplankton cells but leave them essentially intact; they include lime and alum. Results from both studies were consistent in that treatment with Reglone A removed phytoplankton (primarily cyanobacteria) blooms with a concomitant increase in dissolved MCYST (exo-MCYST) concentration in the surrounding water. In contrast, the lime and/or alum treatments (when lake water pH was maintained between 7 and 10) removed blooms without a concomitant increase in exo-MCYST concentrations in the surrounding water. A previous 10-d laboratory experiment also showed that addition of lime to toxic phytoplankton blooms resulted in no increase in MCLR concentration in the water when compared to the control. The integrity of a chemically-treated *Microcystis aeruginosa* culture was examined by both scanning electron microscopy and transmission electron microscopy. Reglone A addition or chlorination caused extensive cell destruction, whereas lime or alum treatments precipitated cells and resulted in minimal cell destruction: the cyanobacterial cells did not rupture and toxin was retained for a substantial period (>26 d) in the laboratory setting. Thus, results from both laboratory and in-situ experiments indicate that the use of chemicals such as lime/alum that precipitate out intact phytoplankton cells can lessen the potential health risk where microcystins are present.

(3) *Persistence of microcystin in lake water*

The persistence of the released microcystin (exo-MCYST) after Reglone A treatment was determined in both the laboratory and lake settings. The estimated half-life of released MCLR from concentrated cyanobacterial blooms in the laboratory setting ranged from 0.5 (±0.1) to 1.6 (±0.0 d). However, no apparent exo-MCYST degradation occurred for ≥ 5d in the closed-bottom limnocorrals in a lake setting. Based on results from two previous studies on the stability of MCLR, MCLR degradation via hydrolysis (Wannamacher 1989) and photodecomposition pathways (Tsuji et al. 1994) would have a minor role in both the present laboratory and in-situ experiments. As MCLR does not partition on lake sediments (Chapter IV) and is susceptible to biotransformation after a 7-10-d lag period (Jones et al. in press; Chapter V), decomposition of exo-MCYST in a lake is likely to occur via biotransformation. The limnocorral study also suggested that exo-MCYST might have a lag-period of ≥ 5d. Thus, the lag-period of biotransformation of exo-MCYSYT in both the laboratory and lake settings is similar.

The present results on biotransformation of MCLR suggested that microbes from wastewater effluent can be induced to transform MCLR; high microbe densities appear to play a role in regulating the MCLR transformation rate. This may in part explain the different reported results on biotransformation of MCYST in the literature (Kiviranta et al. 1991; Jones et al. in press). The transformed product(s) of MCLR, unlike the parent toxin, did not inhibit protein phosphatase activity. Based on first-order kinetics, the biotransformation rate of MCLR ranged from 0.2 to 3.6 d when the microbial population orginating from wastewater effluent was in the order of 10^5 CFU·mL^{-1} (Chapter V). Similar density of microbe population was recorded in lake water when dense phytoplankton collapsed after chemical treatment in the laboratory setting (Lam et al. in press). The limnocorrals study however, suggested that exo-MCYST might have a half-life of ≥ 5d. The biotransformation rate of exo-MCYST was expected to be longer in lakes than in laboratory experiments; the high phytoplankton biomass used in laboratory experiments and other features of the laboratory setting may have resulted in a microbial density which enhanced biotransformation rates over the lake setting.

(4) *Human health implications*

i) Although MCLR concentration is strongly correlated with the volume of *Microcystis* in lakes, however, the production of MCLR by *Microcystis* cells varied over the lifetime of a bloom. Thus, direct monitoring of MCLR concentrations in drinking water lakes is needed for health risk assessments.

ii) Any sudden release of MCYST into surrounding water can present a significant hazard to livestock and human using the water for consumption. The half-life exo-MCYST appears to vary with the intensity of a phytoplankton bloom. At a half-life of $\geq$ 5 d, it would take at least 3 weeks for 90% or over one month for 99% of the released toxin to be degraded. Exposure to substantial sublethal dosages of MCYST through drinking water may cause chronic human health problems (Carmichael and Falconer 1993).

iii) Reglone A or Simazine result in virtually total release of toxin when used to control microcystin-containing cyanobacterial blooms. An appropriate waiting period following the application of these chemicals is necessary before water can be used for consumption. Chlorination and potassium permanganate may not be suitable pre-oxidants for water treatment processes when water is taken from a cyanobacterial-prone source unless the cells are removed prior to the pre-oxidation step. However, lime and/or alum treatments (with pH from 7-10) appear to control the cyanobacterial bloom mainly by cell-coagulation and sedimentation, and are perferable to algicides and chlorination for the control of toxic blooms of cyanobacteria.

iv) As MCLR is susceptible to biotransformation, and its biotransformed product(s) does not inhibit protein phosphatase, it appears that biotransformation can be used as a means for detoxifying MCLR. However, the apparent lag period in biotransformation may prevent this mechanism from contributing to drinking water treatment.

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VII. Appendix A:

Phytoplankton counts, toxin and nutrient data for Little Beaver, Coal, and Driedmeat lakes from 1991-1993

Table A1. Seasonal changes of the abundance (10^6 $\mu m^3 \cdot mL^{-1}$ or $mm^3 \cdot L^{-1}$) of major phytoplankton taxa in Little Beaver Lake from 1991-1993. Surface grab (0-10 cm) water samples were collected in 1991, and compositie (0-1 m) samples were used for both 1992 and 1993.

a) 1991

Date	Cyanobacteria	Chlorophytes	Cryptophytes	Bacillariophytes
Jun-04-91	14.001	0.009	0.071	0.000
Jun-18-91	14.246	0.348	0.366	0.008
Jul-03-91	19.745	0.196	0.077	0.000
Jul-16-91	84.311	0.000	1.123	0.000
Aug-07-91	14.328	0.000	0.008	0.000
Aug-13-91	60.176	0.000	0.018	0.000
Aug-26-91	74.035	0.069	0.541	0.000
Sep-12-91	41.838	1.245	0.886	0.000
Sep-18-91	13.211	0.237	0.122	1.444
Sep-25-91	7.781	0.224	0.131	1.739
Oct-02-91	3.527	0.277	0.073	2.074
Oct-09-91	7.703	0.127	0.154	0.997
Oct-15-91	10.015	0.219	0.791	0.358

b) 1992

Date	Cyanobacteria	Chlorophytes	Cryptophytes	Bacillariophytes	Euglenophytes
May-12-92	0.964	1.596	1.894	1.325	0.000
May-27-92	2.564	0.653	0.501	1.157	0.000
Jun-02-92	3.830	0.644	0.000	0.872	0.000
Jun-09-92	1.305	0.094	0.139	0.039	0.000
Jun-15-92	0.157	0.033	0.000	0.015	0.000
Jun-24-92	1.222	0.126	1.403	0.000	0.000
Jun-30-92	1.696	0.041	0.168	0.000	0.000
Jul-07-92	3.824	0.232	0.304	0.000	0.000
Jul-14-92	5.550	0.231	0.302	0.045	0.000
Jul-21-92	13.109	0.257	0.470	1.062	0.000
Jul-28-92	18.588	0.160	0.054	9.552	0.000
Aug-04-92	17.149	0.421	0.083	13.036	1.307
Aug-10-92	12.149	2.436	0.054	28.251	0.195
Aug-17-92	16.069	1.680	0.027	3.130	0.000
Aug-25-92	10.365	3.568	0.000	3.936	0.000
Sep-03-92	8.704	2.044	0.119	1.158	0.000
Sep-08-92	12.249	2.081	0.056	2.812	0.000
Sep-15-92	19.722	2.396	0.059	5.331	0.000
Sep-21-92	9.995	2.813	0.094	3.704	0.000
Sep-30-92	18.649	2.879	1.007	4.320	0.000

Table A1. (cont.)

c) 1993

Date	Cyanobacteria	Chlorophytes	Cryptophytes	Bacillariophytes
10-May-93	0.774	2.386	0.804	0.639
25-May-93	1.113	1.369	0.044	0.997
08-Jun-93	0.708	0.720	0.041	0.000
24-Jun-93	5.173	1.917	0.461	0.159
07-Jul-93	8.847	10.604	0.366	0.362
12-Jul-93	1.391	1.449	0.000	0.412
20-Jul-93	1.632	1.242	0.438	0.457
27-Jul-93	3.805	1.396	0.888	2.690
04-Aug-93	0.605	0.310	0.198	0.410
10-Aug-93	0.120	1.058	1.184	0.000
17-Aug-93	0.224	0.740	0.000	0.000
24-Aug-93	0.173	0.172	2.987	0.152

Table A2. Seasonal changes of the abundance (10^6 $\mu m^3 \cdot mL^{-1}$ or $mm^3 \cdot L^{-1}$) of major phytoplankton taxa in Coal Lake from 1991-1993. Surface grab (0-10 cm) water samples were collected in 1991, and compositie (0-1 m) samples were used for both 1992 and 1993.

a) 1991

Date	Cyanobacteria	Chlorophytes	Cryptophytes	Bacillariophytes
Jun-05-91	0.430	0.019	0.168	0.131
Jun-19-91	0.828	0.010	1.178	0.053
Jul-04-91	2.051	0.000	0.068	0.059
Jul-17-91	12.674	0.042	0.000	0.000
Jul-25-91	6.866	0.196	0.000	0.000
Aug-08-91	43.377	0.040	0.010	0.000
Aug-12-91	25.785	0.000	0.000	0.000
Aug-21-91	19.699	0.097	0.000	0.000
Aug-28-91	23.611	0.042	0.024	44.866
Sep-12-91	10.999	0.031	0.429	57.865
Sep-18-91	21.753	0.000	0.045	54.184
Sep-25-91	41.519	0.000	0.000	0.000
Oct-02-91	5.006	0.082	0.008	17.667
Oct-09-91	3.312	0.000	0.338	0.000
Oct-15-91	22.414	0.062	0.020	16.302

b) 1992

Date	Cyanobacteria	Chlorophytes	Cryptophytes	Bacillariophytes
May-14-92	0.013	0.017	0.620	0.598
May-28-92	0.080	0.207	1.721	0.000
Jun-03-92	0.010	0.038	0.760	0.021
Jun-10-92	0.044	0.387	0.867	0.269
Jun-16-92	0.109	0.003	1.199	0.000
Jun-25-92	0.292	0.005	0.660	0.942
Jul-02-92	1.579	0.043	0.237	0.661
Jul-08-92	6.135	0.052	0.097	6.235
Jul-15-92	6.175	0.034	0.290	24.845
Jul-22-92	9.828	0.021	0.105	23.831
Jul-29-92	20.178	0.032	0.463	13.877
Aug-06-92	48.610	0.046	0.405	0.000
Aug-11-92	22.400	0.051	0.205	3.835
Aug-18-92	50.627	0.462	0.377	0.000
Aug-26-92	14.793	0.459	0.358	0.000
Aug-31-92	8.723	0.622	1.032	8.959
Sep-08-92	8.022	0.000	0.010	0.000
Sep-16-92	7.779	0.011	0.048	13.227
Sep-22-92	6.438	0.048	0.045	22.774
Sep-29-92	5.716	0.028	0.279	0.000

Table A2. (cont.)

c) 1993

Date	Cyanobacteria	Chlorophytes	Cryptophytes	Bacillariophytes
10-May-93	0.081	0.169	2.023	5.721
25-May-93	0.000	0.111	0.162	0.000
08-Jun-93	0.023	0.054	0.667	3.531
24-Jun-93	0.090	0.016	0.228	0.000
07-Jul-93	1.040	0.063	0.986	0.000
12-Jul-93	2.027	0.036	0.225	0.000
20-Jul-93	4.601	0.131	0.230	0.000
27-Jul-93	4.392	0.112	0.119	0.323
04-Aug-93	7.767	0.087	0.079	0.697
10-Aug-93	5.474	0.040	0.026	0.568
17-Aug-93	7.914	0.131	0.034	0.232
24-Aug-93	0.946	0.035	0.136	0.000

Table A3. Seasonal changes of the abundance (10^6 $\mu m^3 \cdot mL^{-1}$ or $mm^3 \cdot L^{-1}$) of major phytoplankton taxa in Driedmeat Lake from 1991-1993. Surface grab (0-10 cm) water samples were collected in 1991, and compositie (0-1 m) samples were used for both 1992 and 1993.

a) 1991

Date	Cyanobacteria	Chlorophytes	Cryptophytes	Bacillariophytes	Euglenophytes
Jun-14-91	0.018	0.012	1.192	0.026	0.000
Jun-18-91	0.116	0.014	0.501	0.000	0.000
Jul-03-91	0.116	0.088	0.739	0.000	0.000
Jul-16-91	4.609	0.006	0.000	0.000	0.000
Jul-25-91	0.503	1.458	0.000	0.000	0.031
Aug-07-91	16.693	0.000	0.000	0.000	0.000
Aug-13-91	4.774	0.000	0.397	0.000	0.000
Aug-26-91	10.934	0.048	0.000	4.593	0.000
Sep-12-91	45.670	0.019	0.072	16.400	0.000
Sep-18-91	3.039	0.000	0.469	34.720	0.000
Sep-25-91	0.848	0.098	0.013	135.869	13.911
Oct-02-91	1.048	0.343	0.914	12.259	0.000
Oct-09-91	0.060	0.299	0.049	18.392	0.000
Oct-15-91	0.067	0.091	0.021	7.807	0.046

b) 1992

Date	Cyanobacteria	Chlorophytes	Cryptophytes	Bacillariophytes
May-12-92	0.299	0.057	0.778	10.077
May-27-92	0.600	0.070	0.176	0.366
Jun-02-92	0.767	0.169	0.327	0.116
Jun-09-92	0.908	0.025	0.243	1.124
Jun-15-92	1.359	0.002	4.313	0.000
Jun-24-92	28.845	0.216	0.328	0.000
Jun-30-92	28.929	0.034	0.180	0.000
Jul-07-92	83.160	0.076	0.114	0.046
Jul-14-92	53.657	0.007	0.057	0.000
Jul-21-92	249.107	0.025	0.027	0.000
Jul-28-92	43.884	0.025	0.045	0.000
Aug-04-92	20.315	0.000	0.031	0.000
Aug-10-92	30.630	0.000	0.053	0.000
Aug-17-92	31.280	0.000	0.069	0.000
Aug-25-92	17.294	0.210	0.050	0.000
Aug-31-92	21.531	0.000	0.152	0.000
Sep-09-92	15.363	0.123	0.137	0.266
Sep-15-92	9.138	0.048	0.718	2.052
Sep-21-92	5.301	0.014	0.488	4.767
Sep-30-92	0.598	0.203	1.437	19.103

Table A3. (cont.)

c) 1993

Date	Cyanobacteria	Chlorophytes	Cryptophytes	Bacillariophytes
May-10-93	0.556	0.091	7.610	28.422
May-25-93	0.146	0.110	0.102	0.323
Jun-08-93	0.361	0.031	0.883	0.141
Jun-24-93	12.521	0.080	0.153	6.838
Jul-07-93	18.572	0.106	1.320	0.508
Jul-12-93	11.197	0.153	2.013	0.382
Jul-20-93	20.408	0.019	0.068	0.000
Jul-27-93	33.575	0.000	0.036	0.000
Aug-04-93	54.353	0.000	0.197	0.000
Aug-10-93	26.526	0.000	0.000	0.000
Aug-17-93	9.061	0.000	0.044	0.057
Aug-24-93	20.349	0.000	0.000	0.415
Aug-30-93	11.070	0.101	0.132	0.425
Sep-09-93	31.330	0.105	0.711	37.681
Sep-15-93	8.629	0.359	0.187	3.018
Sep-23-93	13.152	0.152	0.498	170.903

Table A4. Relative proportion (by cell volume) of major genera of cyanobacteria in Little Beaver Lake from 1991-1993. Surface grab (0-10 cm) water samples were collected in 1991, and compositie (0-1 m) samples were used for both 1992 and 1993. Note that *Micro.* = *Microcystis* spp.; *Gomp.* = *Gomphosphaeria* sp.; *Ana.* = *Anabaena* spp.; *Aph.* = *Aphanizomenon flos-aquae*; *Phor.* = *Phormidium* sp.; *Meris.* = *Meriomopodia* sp.; *Chro.* = *Chroococcus* spp.; *Coel.* = *Coelosphaeria* spp.; *Aphcap.* = *Aphanocapsa* spp.; *Lyn.* = *Lyngbya* sp.

a) 1991

Date	*Micro.*	*Gomp.*	*Ana.*	*Aph.*	*Phor.*	*Meris.*
Jun-04-91	0.2	0.0	1.6	97.6	0.0	0.0
Jun-18-91	0.0	0.0	12.7	82.5	0.0	0.0
Jul-03-91	0.4	0.5	87.1	11.0	0.0	0.0
Jul-16-91	74.1	1.1	2.7	20.7	0.0	0.0
Aug-07-91	82.2	0.0	0.0	1.8	16.0	0.0
Aug-13-91	51.3	0.0	0.0	43.6	2.5	0.0
Aug-26-91	61.6	0.0	1.3	32.9	3.5	0.0
Sep-12-91	19.4	0.0	0.3	19.4	1.6	0.0
Sep-18-91	64.3	0.3	5.3	17.1	1.0	0.0
Sep-25-91	44.8	3.5	4.6	24.9	1.1	0.0
Oct-02-91	45.4	0.1	0.9	2.7	10.0	0.3
Oct-09-91	50.9	6.0	0.9	6.8	21.1	0.2
Oct-15-91	47.6	0.0	0.9	32.2	6.2	0.0

b) 1992

Date	*Micro.*	*Gomp.*	*Ana.*	*Aph.*	*Phor.*	*Meris.*	*Chro.*	*Coel.*
May-12-92	14.7	0.0	1.5	0.0	0.2	0.3	0.0	0.0
May-27-92	39.8	0.0	11.8	0.0	0.1	0.9	0.0	0.0
Jun-02-92	33.4	0.0	36.5	0.0	1.5	0.2	0.0	0.0
Jun-09-92	48.5	0.0	31.3	0.0	2.7	0.2	0.0	0.0
Jun-15-92	70.9	0.0	0.0	0.0	5.7	0.0	0.0	0.0
Jun-24-92	41.6	0.5	0.0	1.0	1.4	0.0	0.0	0.0
Jun-30-92	88.9	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Jul-07-92	70.9	0.0	0.0	16.8	0.0	0.0	0.0	0.0
Jul-14-92	78.4	0.0	0.0	12.0	0.2	0.0	0.0	0.0
Jul-21-92	85.4	0.0	0.0	2.3	0.3	0.0	0.0	0.0
Jul-28-92	42.2	0.0	0.0	23.1	0.2	0.0	0.0	0.0
Aug-04-92	28.0	0.0	0.0	24.4	1.1	0.1	0.0	0.0
Aug-10-92	24.8	0.0	0.0	0.4	0.5	0.9	0.0	0.0
Aug-17-92	35.1	0.0	16.2	21.7	0.6	3.4	0.0	0.0
Aug-25-92	35.9	0.0	16.6	0.0	0.3	5.3	0.0	0.0
Sep-03-92	39.4	0.0	17.1	4.1	0.2	11.6	0.0	0.0
Sep-08-92	61.6	0.0	0.0	0.2	0.3	9.1	0.0	0.0
Sep-15-92	12.0	0.0	0.0	0.0	0.0	10.2	48.5	1.0
Sep-21-92	12.4	0.0	0.0	0.7	0.0	6.0	39.3	1.6
Sep-30-92	2.3	0.0	0.0	0.0	0.1	2.6	64.1	0.4

Table A4. (cont.)

c) 1993

Date	*Micro.*	*Gomp.*	*Ana.*	*Aph.*	*Phor.*	*Meris.*	*Aphcap.*
10-May-93	8.5	0.0	8.3	0.0	0.0	0.0	0.0
25-May-93	12.7	0.0	18.5	0.0	0.0	0.4	0.0
08-Jun-93	41.5	0.0	2.9	0.0	0.0	3.8	0.0
24-Jun-93	16.4	0.0	49.3	0.0	0.0	1.3	0.0
07-Jul-93	3.0	0.0	40.2	0.0	0.0	0.6	0.0
12-Jul-93	3.1	0.0	35.0	0.0	0.0	3.4	1.2
20-Jul-93	2.2	0.0	41.1	0.0	0.0	0.0	0.0
27-Jul-93	1.4	0.0	0.0	39.3	0.0	0.3	2.4
04-Aug-93	13.3	0.0	21.6	0.0	0.0	0.7	4.1
10-Aug-93	4.0	0.0	0.0	0.0	0.0	0.7	0.4
17-Aug-93	22.0	0.0	0.0	0.0	0.0	0.0	1.3
24-Aug-93	3.4	0.0	0.0	0.0	0.0	0.0	1.6

Table A5. Relative proportion (by cell volume) of major genera of cyanobacteria in Coal Lake from 1991-1993. Surface grab (0-10 cm) water samples were collected in 1991, and compositie (0-1 m) samples were used for both 1992 and 1993.

a) 1991

Date	*Micro.*	*Gomp.*	*Ana.*	*Aph.*	*Phor.*
Jun-05-91	54.7	2.3	0.0	0.0	0.6
Jun-19-91	16.2	1.7	18.0	0.0	4.1
Jul-04-91	10.5	16.2	0.0	67.4	0.0
Jul-17-91	14.0	25.8	0.0	59.2	0.6
Jul-25-91	52.1	36.3	0.0	7.7	1.2
Aug-08-91	4.1	13.5	0.0	82.2	0.0
Aug-12-91	5.2	13.8	0.0	80.9	0.1
Aug-21-91	14.1	15.6	0.0	69.3	0.6
Aug-28-91	6.0	6.8	0.0	21.6	0.1
Sep-12-91	3.3	9.9	0.0	2.6	0.0
Sep-18-91	2.3	23.2	0.0	3.1	0.0
Sep-25-91	8.2	24.0	0.0	67.9	0.0
Oct-02-91	2.1	15.8	0.0	4.1	0.0
Oct-09-91	15.1	46.4	0.0	28.8	0.4
Oct-15-91	3.0	19.4	0.0	35.4	0.0

b) 1992

Date	*Micro.*	*Gomp.*	*Ana.*	*Aph.*	*Phor.*	*Lyn.*	*Meris.*
May-14-92	0.0	0.8	0.0	0.0	0.3	0.0	0.0
May-28-92	0.0	4.0	0.0	0.0	0.0	0.0	0.0
Jun-03-92	0.0	1.2	0.0	0.0	0.0	0.0	0.0
Jun-10-92	0.0	0.6	2.2	0.0	0.0	0.0	0.0
Jun-16-92	1.3	3.9	3.1	0.0	0.0	0.0	0.0
Jun-25-92	4.6	6.7	1.1	2.9	0.0	0.0	0.0
Jul-02-92	1.3	10.4	3.2	47.4	0.3	0.0	0.0
Jul-08-92	2.3	5.3	3.5	38.1	0.2	6.6	0.0
Jul-15-92	1.3	3.3	4.8	10.2	0.1	2.2	0.0
Jul-22-92	0.5	3.2	0.1	25.3	0.0	0.0	0.0
Jul-29-92	0.5	6.7	0.0	51.1	0.1	2.6	0.0
Aug-06-92	0.7	11.2	0.0	87.1	0.0	0.1	0.0
Aug-11-92	0.7	12.3	0.0	71.5	0.0	0.0	0.0
Aug-18-92	1.3	21.4	0.0	75.6	0.0	0.0	0.0
Aug-26-92	3.0	34.2	0.0	57.4	0.1	0.0	0.0
Aug-31-92	1.8	34.9	0.0	8.4	0.0	0.0	0.0
Sep-08-92	2.4	93.6	2.6	1.2	0.0	0.0	0.0
Sep-16-92	0.5	35.7	0.0	0.8	0.0	0.0	0.0
Sep-22-92	0.1	19.1	0.0	2.7	0.0	0.0	0.0
Sep-29-92	0.0	94.9	0.0	0.0	0.0	0.0	0.0

Table A2. (cont.)

c) 1993

Date	*Micro.*	*Gomp.*	*Ana.*	*Aph.*	*Lyn.*	*Meris.*
10-May-93	1.0	0.0	0.0	0.0	0.0	0.0
25-May-93	0.0	0.0	0.0	0.0	0.0	0.0
08-Jun-93	0.0	0.5	0.0	0.0	0.0	0.0
24-Jun-93	2.5	6.8	17.6	0.0	0.0	0.0
07-Jul-93	0.7	0.0	49.1	0.0	0.0	0.0
12-Jul-93	0.0	0.7	0.0	36.9	51.0	0.0
20-Jul-93	0.5	0.6	24.3	27.1	40.2	0.0
27-Jul-93	0.0	0.9	0.0	0.0	87.9	0.0
04-Aug-93	0.2	0.0	0.2	22.7	67.0	0.0
10-Aug-93	0.0	0.0	2.3	0.0	87.3	0.0
17-Aug-93	0.2	0.0	2.5	13.2	79.4	0.0
24-Aug-93	0.0	0.0	0.0	84.7	0.0	0.0

Table A6. Relative proportion (by cell volume) of major genera of cyanobacteria in Driedmeat Lake from 1991-1993. Surface grab (0-10 cm) water samples were collected in 1991, and compositie (0-1 m) samples were used for both 1992 and 1993.

a) 1991

Date	*Micro.*	*Gomp.*	*Ana.*	*Aph.*	*Phor.*
Jun-14-91	0.7	0.8	0.0	0.0	0.0
Jun-18-91	16.9	1.6	0.0	0.0	0.0
Jul-03-91	12.3	0.0	0.0	0.0	0.0
Jul-16-91	4.9	0.4	87.3	6.7	0.6
Jul-25-91	5.2	0.5	3.8	15.8	0.0
Aug-07-91	7.3	3.3	0.4	88.8	0.2
Aug-13-91	14.9	5.5	0.0	71.6	0.3
Aug-26-91	15.0	1.0	12.2	41.7	0.4
Sep-12-91	2.8	0.3	1.1	69.4	0.0
Sep-18-91	3.2	0.4	0.7	3.7	0.0
Sep-25-91	0.4	0.1	0.0	0.0	0.1
Oct-02-91	3.5	0.8	2.9	0.0	0.0
Oct-09-91	0.2	0.1	0.0	0.0	0.0
Oct-15-91	0.5	0.4	0.0	0.0	0.0

b) 1992

Date	*Micro.*	*Gomp.*	*Ana.*	*Aph.*	*Phor.*
May-12-92	0.8	0.0	1.8	0.0	0.0
May-27-92	0.2	0.0	49.3	0.0	0.0
Jun-02-92	0.0	0.0	2.9	52.8	0.0
Jun-09-92	0.0	0.5	7.4	31.6	0.0
Jun-15-92	0.0	0.0	23.9	0.0	0.1
Jun-24-92	0.8	0.0	5.1	92.3	0.0
Jun-30-92	0.4	0.0	0.3	98.5	0.0
Jul-07-92	1.1	0.0	0.3	98.2	0.1
Jul-14-92	6.3	0.0	0.0	93.4	0.2
Jul-21-92	4.1	0.0	0.0	95.6	0.2
Jul-28-92	6.8	0.0	0.0	91.8	1.2
Aug-04-92	38.7	0.0	0.0	58.4	2.8
Aug-10-92	40.0	0.0	0.0	56.4	3.4
Aug-17-92	36.1	0.0	0.0	61.6	2.1
Aug-25-92	57.3	0.0	0.0	37.3	3.9
Aug-31-92	28.0	0.0	0.0	68.9	1.5
Sep-09-92	57.6	0.1	0.0	35.9	3.1
Sep-15-92	26.0	0.0	0.0	49.3	1.2
Sep-21-92	11.8	0.0	0.0	37.8	0.6
Sep-30-92	0.4	0.0	0.0	2.2	0.2

Table A6. (cont.)

c) 1993

Date	*Micro.*	*Gomp.*	*Ana*	*Aph.*	*Phor.*	*Meris.*
May-10-93	0.4	0.0	1.1	0.0	0.0	0.0
May-25-93	17.1	0.0	0.0	4.4	0.0	0.0
Jun-08-93	17.4	0.0	3.6	0.0	4.5	0.0
Jun-24-93	4.0	0.0	0.0	59.8	0.1	0.0
Jul-07-93	11.6	0.0	4.7	74.0	0.0	0.0
Jul-12-93	20.7	0.0	6.7	52.2	1.9	0.0
Jul-20-93	17.2	0.0	3.2	77.5	1.6	0.0
Jul-27-93	34.7	0.2	1.0	62.3	1.8	0.0
Aug-04-93	57.2	0.2	0.0	41.4	0.8	0.0
Aug-10-93	44.6	0.3	0.0	53.3	1.8	0.0
Aug-17-93	55.8	1.2	5.3	34.7	2.0	0.0
Aug-24-93	35.4	0.2	0.0	61.2	1.2	0.0
Aug-30-93	33.5	0.4	0.0	60.2	0.3	0.0
Sep-09-93	28.9	0.1	0.0	15.7	0.1	0.0
Sep-15-93	17.4	0.7	0.0	52.1	0.4	0.2
Sep-23-93	3.8	0.1	0.0	3.3	0.0	0.0

Table A7. Intracellular microcystin-LR concentrations, expressed as both $\mu g \cdot g^{-1}$ biomass and $\mu g \cdot cell^{-1}$ based on chlorophyll *a* concentrations, in the three study lakes from 1991-93. 'N/D' = non-detectable MCLR concentration; 'N/S' = no sample; 'N/G' = no chlorophyll *a* data; 'N/C' = no detectable *Microcystis* cell.

a) Little Beaver Lake

1991	Endo-MCLR ($\mu g \cdot g^{-1}$)	Endo-MCLR ($pg \cdot cell^{-1}$)	1992	Endo-MCLR ($\mu g \cdot g^{-1}$)	Endo-MCLR ($pg \cdot cell^{-1}$)	1993	Endo-MCLR ($\mu g \cdot g^{-1}$)	Endo-MCLR ($pg \cdot cell^{-1}$)
Jun-04-91	0	0	May-12-92	865	0.283	10-May-93	101	0.138
Jun-18-91	18	0.182	May-27-92	N/D	0	25-May-93	110	0.046
Jul-03-91	84	0.634	Jun-02-92	429	0.052	08-Jun-93	64	0.019
Jul-16-91	474	0.010	Jun-09-92	195	0.008	24-Jun-93	46	0.015
Aug-07-91	915	0.034	Jun-15-92	21	0.002	07-Jul-93	N/D	0
Aug-13-91	923	0.021	Jun-24-92	107	0.005	12-Jul-93	N/D	0
Aug-26-91	800	0.027	Jun-30-92	255	0.009	20-Jul-93	29	0.091
Sep-12-91	815	0.029	Jul-07-92	205	0.006	27-Jul-93	N/D	0
Sep-18-91	798	0.074	Jul-14-92	344	0.008	04-Aug-93	N/D	0
Sep-25-91	711	0.096	Jul-21-92	389	0.008	10-Aug-93	38	0.056
Oct-02-91	903	0.160	Jul-28-92	234	0.005	17-Aug-93	N/D	0
Oct-09-91	1307	0.124	Aug-04-92	240	0.012	24-Aug-93	30	0.027
Oct-15-91	375	0.064	Aug-10-92	363	N/G			
			Aug-17-92	248	0.025			
			Aug-25-92	184	0.021			
			Sep-03-92	18	N/G			
			Sep-08-92	150	0.012			
			Sep-15-92	54	0.008			
			Sep-21-92	54	0.011			
			Sep-30-92	32	N/G			

Table A7 cont...

b) Coal Lake

1991	Endo-MCLR ($\mu g \cdot g^{-1}$)	Endo-MCLR ($pg \cdot cell^{-1}$)	1992	Endo-MCLR ($\mu g \cdot g^{-1}$)	Endo-MCLR ($pg \cdot cell^{-1}$)	1993	Endo-MCLR ($\mu g \cdot g^{-1}$)	Endo-MCLR ($pg \cdot cell^{-1}$)
Jun-05-91	105	0.007	May-14-92	N/S	N/S	10-May-93	N/D	0
Jun-19-91	93	0.035	May-28-92	N/D	N/C	25-May-93	N/D	0
Jul-04-91	N/D	N/D	Jun-03-92	N/D	N/C	08-Jun-93	50	0
Jul-17-91	N/D	N/D	Jun-10-92	N/D	N/C	24-Jun-93	19	0.003
Jul-25-91	N/D	N/D	Jun-16-92	1	0	07-Jul-93	N/D	0
Aug-08-91	42	0.016	Jun-25-92	N/D	N/D	12-Jul-93	21	N/C
Aug-12-91	20	0.004	Jul-02-92	N/D	N/D	20-Jul-93	21	0
Aug-21-91	231	0.035	Jul-08-92	N/D	N/D	27-Jul-93	N/D	N/C
Aug-28-91	229	0.027	Jul-15-92	N/D	N/D	04-Aug-93	22	0.002
Sep-12-91	143	0.022	Jul-22-92	N/D	N/D	10-Aug-93	18	0
Sep-18-91	161	0.031	Jul-29-92	N/D	N/D	17-Aug-93	23	0.002
Sep-25-91	185	0.016	Aug-06-92	N/D	N/D	24-Aug-93	19	0
Oct-02-91	N/S	N/S	Aug-11-92	N/D	N/D			
Oct-09-91	40	0.011	Aug-18-92	N/D	N/D			
Oct-15-91	16	0.002	Aug-26-92	N/D	N/D			
			Aug-31-92	N/S	N/S			
			Sep-08-92	N/D	N/D			
			Sep-16-92	N/D	N/D			
			Sep-22-92	N/S	N/S			
			Sep-29-92	N/D	N/D			

Table A7 cont...

c) Driedmeat Lake

1991	Endo-MCLR ($\mu g \cdot g^{-1}$)	Endo-MCLR ($pg \cdot cell^{-1}$)	1992	Endo-MCLR ($\mu g \cdot g^{-1}$)	Endo-MCLR ($pg \cdot cell^{-1}$)	1993	Endo-MCLR ($\mu g \cdot g^{-1}$)	Endo-MCLR ($pg \cdot cell^{-1}$)
Jun-14-91	N/S	N/S	May-12-92	14	0	May-10-93	77	0.372
Jun-18-91	N/S	N/S	May-27-92	N/S	N/S	May-25-93	71	0.017
Jul-03-91	722	0.023	Jun-02-92	N/D	N/D	Jun-08-93	24	0.005
Jul-16-91	N/S	N/S	Jun-09-92	N/D	N/D	Jun-24-93	26	0.011
Jul-25-91	194	0.003	Jun-15-92	5	N/D	Jul-07-93	143	0.034
Aug-07-91	45	0	Jun-24-92	N/D	N/D	Jul-12-93	191	N/G
Aug-13-91	124	0	Jun-30-92	N/D	N/D	Jul-20-93	218	0.050
Aug-26-91	346	0	Jul-07-92	31	0	Jul-27-93	431	0.028
Sep-12-91	95	0	Jul-14-92	129	0	Aug-04-93	414	0.010
Sep-18-91	209	0	Jul-21-92	107	0	Aug-10-93	463	0.023
Sep-25-91	340	0	Jul-28-92	137	0	Aug-17-93	571	0.040
Oct-02-91	N/S	N/S	Aug-04-92	563	0	Aug-24-93	533	0.034
Oct-09-91	N/S	N/S	Aug-10-92	588	0	Aug-30-93	629	0.078
Oct-15-91	N/S	N/S	Aug-17-92	705	0	Sep-09-93	558	0.011
			Aug-25-92	938	0	Sep-15-93	813	0.154
			Aug-31-92	699	0	Sep-23-93	331	0.012
			Sep-09-92	779	0			
			Sep-15-92	232	0			
			Sep-21-92	211	0			
			Sep-30-92	84	0			

Table A8. Nutrient concentrations collected from the three study lakes from 1991-1993. 'N/D' = non-detectable and 'N/S' = missing sample.

a) Little Beaver Lake

1991	Chl*a* $\mu g \cdot L^{-1}$	NO_3^- $\mu g \cdot L^{-1}$	NH_4^+ $\mu g \cdot L^{-1}$	TP $\mu g \cdot L^{-1}$	TDP $\mu g \cdot L^{-1}$	CO_3^{2-} $mg \cdot L^{-1}$	HCO_3^- $mg \cdot L^{-1}$	pH	WTemp °C	Secchi m
Jun-04-91	10.2	156	22.6	115	86	17.4	365	8.7	17.5	2.2
Jun-18-91	29.0	11.5	134	94	38	38.9	319	9.0	16.3	1.8
Jul-03-91	16.9	30.5	104	127	71	24.3	328	8.9	23.2	2
Jul-16-91	90.9	13.8	14.2	279	129	59.1	262	9.4	25	0.9
Aug-07-91	34.4	8.5	17.3	241	136	70.6	235	9.4	21.8	1.2
Aug-13-91	55.0	12.6	41.6	244	138	76.1	204	9.6	21.2	1
Aug-26-91	110	2.7	12.3	198	74	81.9	187	9.7	18.8	0.7
Sep-12-91	65.5	4.7	44.3	171	58	59.2	224	9.6	14.6	0.4
Sep-18-91	82.9	9.6	28.1	N/S	N/S	57.4	234	9.5	10.6	0.3
Sep-25-91	100	0.9	20.6	183	68	0	70	9.6	11.6	0.2
Oct-02-91	86.8	6	12.9	169	74	75.9	192	9.7	10	0.5
Oct-09-91	70.5	107	6.7	141	54	72.5	214	9.7	8	0.4
Oct-15-91	76.8	142	10.6	166	49	68.9	219	9.6	8.4	0.4

Table A8. cont...

a) Little Beaver Lake (cont.)

1992	Chl*a* $\mu g \cdot L^{-1}$	NH_4^+ $\mu g \cdot L^{-1}$	TP $\mu g \cdot L^{-1}$	TDP $\mu g \cdot L^{-1}$	CO_3^{2-} $mg \cdot L^{-1}$	HCO_3^- $mg \cdot L^{-1}$	pH	WTemp °C	Secchi m
May-12-92	31.4	8.5	159	43	N/S	N/S	9.2	10.3	0.47
May-27-92	16.4	37.6	177	52	41.1	291	9.2	14.3	0.5
Jun-02-92	20.0	2.7	162	45	33.8	297	9.0	16.5	0.65
Jun-09-92	2.8	1111	96	73	27.5	308	9.0	18.3	1.8
Jun-15-92	1.1	521	146	119	16.1	340	8.7	18.2	1.6
Jun-24-92	6.4	736	139	115	N/S	372	8.5	22.5	2
Jun-30-92	5.8	808	149	113	N/S	379	8.2	20.6	2.2
Jul-07-92	8.4	694	149	119	N/S	382	8.3	20	1.9
Jul-14-92	10.9	340	127	80	N/S	390	8.3	19.5	1.38
Jul-21-92	22.4	5.2	128	52	15.2	355	8.7	20	0.9
Jul-28-92	37.5	48.6	167	66	26.4	331	8.9	21.4	0.4
Aug-04-92	62.7	4.1	230	80	33.6	339	9.1	24.4	0.4
Aug-10-92	N/S	3.6	230	43	39.8	312	9.2	20.2	0.4
Aug-17-92	70.3	5.2	246	57	46.4	291	9.2	22.4	0.3
Aug-25-92	71.4	241	53	42	28.7	310	9.0	12.5	0.25
Sep-03-92	N/S	2.8	175	43	N/S	N/S	N/S	13.9	0.35
Sep-08-92	52.0	5.5	242	39	32.9	295	9.1	10.5	0.27
Sep-15-92	44.9	2.2	170	41	30.7	304	9.0	8.5	0.4
Sep-21-92	40.7	44.2	171	37	23.0	302	9.0	9	0.2
Sep-30-92	27.8	0.5	136	45	23.1	331	8.9	10	0.5

1993	Chl*a* $\mu g \cdot L^{-1}$	NO_3^- $\mu g \cdot L^{-1}$	NH_4^+ $\mu g \cdot L^{-1}$	TN $\mu g \cdot L^{-1}$	TP $\mu g \cdot L^{-1}$	TDP $\mu g \cdot L^{-1}$	CO_3^{2-} $mg \cdot L^{-1}$	HCO_3^- $mg \cdot L^{-1}$	pH	WTemp °C	Secchi m
10-May-93	54.8	19.0	66	1657	186	32	17	294	8.6	13.0	0.4
25-May-93	19.2	12.3	18	1116	146	36	20	282	8.7	15.2	0.5
08-Jun-93	16.3	10.4	11	1965	144	50	22	358	8.9	18.7	0.7
24-Jun-93	63.0	4.1	0	1562	200	41	37	356	9.0	12.9	0.4
07-Jul-93	30.2	3.2	78	N/S	314	43	60	287	9.3	18.3	0.4
12-Jul-93	0.0	14.1	N/D	1511	142	42	65	278	9.4	17.7	0.6
20-Jul-93	25.3	10.8	18	1621	120	43	78	271	9.5	18.7	0.6
27-Jul-93	31.9	4.1	26	1731	128	42	82	227	9.5	20.1	0.7
04-Aug-93	17.0	0.9	15	1521	99	45	105	231	9.7	20.1	0.9
10-Aug-93	26.3	11.0	7	1490	128	59	102	238	9.7	19.0	0.9
17-Aug-93	21.7	3.8	30	1718	127	60	107	230	9.8	18.9	0.7
24-Aug-93	23.7	3.7	39	1223	154	78	N/S	N/S	N/S	19.3	0.4

b) Coal Lake

1991	Chl*a* $\mu g \cdot L^{-1}$	NO_3^- $\mu g \cdot L^{-1}$	NH_4^+ $\mu g \cdot L^{-1}$	TP $\mu g \cdot L^{-1}$	TDP $\mu g \cdot L^{-1}$	CO_3^{2-} $mg \cdot L^{-1}$	HCO_3^- $mg \cdot L^{-1}$	pH	WTemp °C	Secchi m
Jun-05-91	5.1	67	161	54	28	0	231	8.3	16.7	2.5
Jun-19-91	14.4	30	27	58	24.5	0	227	7.4	16.8	2.3
Jul-04-91	9.0	38	67	46	26.1	5.5	221	8.6	20.1	3.2
Jul-17-91	29.4	20	10	76	27.3	4.6	218	8.6	21.4	1.5
Jul-25-91	25.6	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.8
Aug-08-91	60.9	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	1.9
Aug-12-91	31.8	202	457	109	58.2	4.7	198	8.6	20.5	1.6
Aug-21-91	43.9	N/S	N/S	192	N/S	N/S	N/S	N/S	N/S	N/S
Aug-28-91	48.4	20	17	182	96	4.9	208	8.5	19.2	0.9
Sep-12-91	35.7	5	13	97	26.5	0	212	8.2	15.3	0.8
Sep-18-91	30.2	2	12	89	28.1	0.7	208	8.3	12.5	0.9
Sep-25-91	26.8	8	32	68	29.6	N/S	N/S	8.2	11.7	1.5
Oct-02-91	16.4	19	15	62	27	9.4	193	8.4	10.9	1
Oct-09-91	13.9	215	22	61	28.1	N/S	217	8.1	8.9	1.5
Oct-15-91	15.4	156	382	61	22.3	N/S	218	8.3	8.8	0.9

1992	Chl*a* $\mu g \cdot L^{-1}$	NH_4^+ $\mu g \cdot L^{-1}$	TP $\mu g \cdot L^{-1}$	TDP $\mu g \cdot L^{-1}$	CO_3^{2-} $mg \cdot L^{-1}$	HCO_3^- $mg \cdot L^{-1}$	pH	WTemp °C	Secchi m
May-14-92	1.6	262	48.8	29.2	N/S	240	8.1	11.7	2.6
May-28-92	3.6	20.5	44.3	24.1	N/S	235	8.0	13.1	2.4
Jun-03-92	3.5	51.6	112	24.5	N/S	214	8.2	14.2	1.4
Jun-10-92	3.7	17.7	63	23.8	N/S	237	8.2	16.7	2.4
Jun-16-92	3.2	35.4	39.8	19	N/S	236	8.1	17.3	2.0
Jun-25-92	3.5	99.3	34.3	19.4	N/S	233	8.4	20.5	1.9
Jul-02-92	7.9	15.7	47.1	22.1	N/S	236	8.3	19.7	2.2
Jul-08-92	16.1	14.5	56.4	25.4	2.4	234	8.5	18.8	2.0
Jul-15-92	22.6	48.8	82.4	20.4	4.6	220	8.5	18.0	0.8
Jul-22-92	24.0	63.1	58.3	18.3	8.5	225	8.7	19.2	0.6
Jul-29-92	20.2	4	48	17.4	8.5	223	8.7	19.9	1.0
Aug-06-92	39.2	10.3	72.9	18.2	13.6	195	8.9	20.9	1.2
Aug-11-92	32.0	9.9	69.7	17.1	10.1	198	8.7	19.8	0.8
Aug-18-92	26.1	92.3	125	39.7	4.7	193	8.6	21.7	1.2
Aug-26-92	23.5	10.9	162	47.4	N/S	N/S	8.6	15.3	1.0
Aug-31-92	23.7	8.2	100	37.2	5.0	194	8.6	14.8	0.8
Sep-08-92	15.1	5.4	93.5	33.4	4.6	195	8.6	12.1	0.7
Sep-16-92	7.6	19.1	72.9	25.7	4.3	193	8.5	9.5	0.8
Sep-22-92	13.1	8.6	62.5	19.9	N/S	208	8.2	11.9	0.8
Sep-29-92	12.4	0.7	63.9	21.7	N/S	207	8.3	9.0	1.0

b) Coal Lake (cont.)

1993	Chl*a* $\mu g \cdot L^{-1}$	NO_3^- $\mu g \cdot L^{-1}$	NH_4^+ $\mu g \cdot L^{-1}$	TN $\mu g \cdot L^{-1}$	TP $\mu g \cdot L^{-1}$	TDP $\mu g \cdot L^{-1}$	CO_3^{2-} $mg \cdot L^{-1}$	HCO_3^- $mg \cdot L^{-1}$	pH	WTemp °C	Secchi m
10-May-93	11.5	9.4	130	874	54.9	20.3	3.9	185	8.3	11.1	1.3
25-May-93	7.0	37.2	85.1	772	42	21.8	N/S	184	8.1	13.9	1.8
08-Jun-93	2.9	15.1	19.5	862	35.1	17.8	1.9	241	8.3	16.9	1.8
24-Jun-93	5.6	8	29.2	886	50.9	28	6.6	236	8.4	15.1	1.4
07-Jul-93	8.1	5.2	61.5	666	42.4	20.3	11.0	223	8.5	17.1	1.6
12-Jul-93	N/S	13.6	N/D	910	45.2	20.7	13.5	218	8.7	17.1	1.2
20-Jul-93	9.8	13.1	21.4	421	44.3	22	12.7	231	8.7	18.2	1.6
27-Jul-93	10.4	4.9	14.2	742	50.1	20.7	14.7	227	8.6	19.3	1.2
04-Aug-93	11.5	5.9	0.2	680	44.3	12.8	19.1	218	8.7	19.6	1.0
10-Aug-93	14.1	13.2	N/D	853	61.5	16.8	13.5	210	8.6	18.7	0.9
17-Aug-93	10.3	6.4	26.7	436	48.8	17.9	20.3	217	8.9	18.7	1.3
24-Aug-93	22.6	4.9	40.9	511	52.9	27.3	N/S	N/S	N/S	19.1	1.4

c) Driedmeat Lake

1991	Chl*a* $\mu g \cdot L^{-1}$	NO_3^- $\mu g \cdot L^{-1}$	NH_4^+ $\mu g \cdot L^{-1}$	TP $\mu g \cdot L^{-1}$	TDP $\mu g \cdot L^{-1}$	pH	WTemp °C	Secchi m
Jun-14-91	6.3	11.8	102	128	92.7	8.3	15.7	2.1
Jun-18-91	5.6	127	42.0	127	87.1	8.2	15.7	3.1
Jul-03-91	4.5	22.7	16.7	127	106	8.5	21.6	3.2
Jul-16-91	6.7	19.3	17.0	174	137	8.9	22	1.9
Jul-25-91	6.4	N/S	N/S	N/S	N/S	N/S	N/S	1.7
Aug-07-91	19.4	57.1	11.7	281	214	8.9	20.8	1.6
Aug-13-91	26.9	23.2	19.5	276	216	9.0	20.5	1.6
Aug-26-91	30.1	6.4	44.7	296	231	9.1	19.1	1.6
Sep-12-91	22.0	8.8	52.7	232	151	8.5	15.1	1.4
Sep-18-91	29.3	2.7	75.9	183	113	8.3	11.2	1.0
Sep-25-91	23.7	2.4	17.9	146	63.2	8.6	11.9	0.5
Oct-02-91	22.4	14.8	10.8	125	41.6	8.4	10.4	0.9
Oct-09-91	19.6	17.6	41.4	83.6	25.6	8.5	8.6	0.9
Oct-15-91	30.3	342	11.4	145	25.4	8.3	8.6	0.5

c) Driedmeat Lake (cont.)

1992	Chl*a* $\mu g \cdot L^{-1}$	NH_4^+ $\mu g \cdot L^{-1}$	TP $\mu g \cdot L^{-1}$	TDP $\mu g \cdot L^{-1}$	CO_3^{2-} $mg \cdot L^{-1}$	HCO_3^- $mg \cdot L^{-1}$	pH	WTemp °C	Secchi m
May-12-92	15.5	35	128	59	N/S	246	8.3	11.4	1.0
May-27-92	5.4	227	153	116	N/S	275	8.2	13	2.2
Jun-02-92	3.9	1078	187	134	N/S	278	8.0	16.2	1.0
Jun-09-92	4.2	1607	140	117	3.2	273	8.4	16.9	2.6
Jun-15-92	5.7	88	148	118	8.0	267	8.5	17.4	2.6
Jun-24-92	38.1	17	132	75	N/S	269	9.0	20.7	1.0
Jun-30-92	46.0	72	202	141	14.2	232	8.8	20	1.1
Jul-07-92	99.9	15	259	159	29.4	189	9.2	18.9	0.3
Jul-14-92	65.6	48	317	232	37.6	172	9.3	18.6	0.9
Jul-21-92	52.9	22	422	287	36.7	169	9.3	18.7	0.7
Jul-28-92	82.9	45	459	414	52.3	133	9.5	19.9	0.3
Aug-04-92	79.2	46	667	597	36.8	146	9.4	22.4	0.5
Aug-10-92	61.3	34	692	641	48.6	143	9.6	19.5	1.0
Aug-17-92	57.0	9	758	600	57.2	118	9.7	20.6	1.2
Aug-25-92	31.4	26	758	715	39.2	156	9.4	13	0.7
Aug-31-92	25.9	12	757	694	36.2	158	9.4	14.8	0.6
Sep-09-92	37.3	79	708	591	28.4	171	9.3	10.7	0.4
Sep-15-92	25.8	88	713	616	22.1	189	9.1	8.8	0.4
Sep-21-92	27.8	100	678	542	19.4	189	9.1	9	0.3
Sep-30-92	34.4	1	517	407	19.4	202	9.0	9.4	0.6

1993	Chl*a* $\mu g \cdot L^{-1}$	NO_3^- $\mu g \cdot L^{-1}$	NH_4^+ $\mu g \cdot L^{-1}$	TN $\mu g \cdot L^{-1}$	TP $\mu g \cdot L^{-1}$	TDP $\mu g \cdot L^{-1}$	CO_3^{2-} $mg \cdot L^{-1}$	HCO_3^- $mg \cdot L^{-1}$	pH	WTemp °C	Secchi m
May-10-93	83	8	195	898	208	33	15	138	8.9	12.7	0.6
May-25-93	3	19	19	780	219	189	5	161	8.4	15.2	1.7
Jun-08-93	6	61	158	1441	286	246	17	238	8.7	18.3	2.4
Jun-24-93	35	83	110	1244	407	294	11	232	8.5	14.2	0.6
Jul-07-93	45	8	201	1042	324	234	29	197	9.1	17.6	1.4
Jul-12-93	0	20	7	1223	334	243	33	189	9.2	16.9	1.3
Jul-20-93	72	12	33	540	401	290	43	181	9.3	18.2	1.1
Jul-27-93	67	9	25	1018	535	443	60	158	9.5	25.9	0.8
Aug-04-93	111	0	21	962	675	501	64	155	9.5	19.2	0.9
Aug-10-93	90	10	15	1033	760	623	59	167	9.5	18.2	0.6
Aug-17-93	55	4	48	578	739	625	71	145	9.7	19.0	0.8
Aug-24-93	72	4	33	691	827	702	N/S	N/S	N/S	18.7	0.5
Aug-30-93	76	7	14	683	817	702	56	175	9.4	15.9	0.5
Sep-09-93	77	8	7	1263	808	624	47	200	9.2	17.2	0.4
Sep-15-93	69	9	89	1495	827	674	47	204	9.2	12.8	0.3
Sep-23-93	58	2	6	2132	745	547	60	195	8.9	10.8	0.4

VII. Appendix B:

Microcystin-LR concentrations, nutrient data, and bacterial densities for the three Batch experiments

Table B1. Concentrations of dissolved microcystin-LR (exo-MCLR), dissolved total microcystins (MCYST), and other chemical parameters in Batch 1. 'N/D' represents non-detectable, and 'lost' represents missing sample.

Treatment	Day	Volume (L)	NO_3^- ($\mu g \cdot L^{-1}$)	TDP ($mg \cdot L^{-1}$)	pH	Exo-MCLR ($mg \cdot L^{-1}$)	Exo-MCLR ($mg \cdot g^{-1}$)	MCYST ($mg \cdot L^{-1}$)
Reglone A	0	12	2.4	0.14	6.67	0.01	0.01	0.001
	1	11.4	3.1	N/D	6.82	0.02	0.03	0.044
	2	10.9	6.1	4.62	6.32	1.37	1.66	3.040
	3	10.4	6.7	6.33	6.29	2.32	2.68	6.660
	4	9.9	17.3	7.05	6.28	2.16	2.37	6.210
	7	9.4	12.4	4.46	6.83	0.49	0.51	0.940
	10	8.9	22.3	12.22	7.30	0.06	0.06	0.420
	14	8.4	9.1	8.27	7.22	0.06	0.06	0.410
	19	7.9	24.0	7.08	7.49	N/D	N/D	lost
	23	7.4	40.7	7.97	8.10	N/D	N/D	0.245
	49	6.9	38.1	N/D	8.40	N/D	N/D	0.285
Simazine	0	12	6.9	0.08	6.97	0	0.00	0.000
	1	11.4	3.2	0.28	6.92	0	0.00	0.007
	2	10.9	3.3	2.70	6.64	0.02	0.01	0.044
	3	10.4	17.3	1.60	6.72	0.11	0.08	0.076
	4	9.9	62.8	1.41	6.75	0.11	0.07	0.057
	7	9.4	3.0	0.71	7.08	0.3	0.19	0.046
	10	8.9	13.7	1.15	7.41	0.22	0.13	0.000
	14	8.4	1.8	0.76	7.37	0.23	0.13	0.007
	19	7.9	7.1	0.28	7.84	N/D	N/D	lost
	23	7.4	27.8	0.62	8.36	N/D	N/D	0.007
	49	6.9	88.9	1.14	8.34	N/D	N/D	0.008
$KMnO_4$	0	12	1.5	0.11	6.73	0.02	0.01	0.000
	1	11.4	5.7	3.23	6.10	0.56	0.24	1.050
	2	10.9	N/D	4.20	5.86	1.12	0.45	2.560
	3	10.4	34.0	5.18	6.06	2.19	0.84	3.060
	4	9.9	126.2	4.74	6.38	0.54	0.20	0.615
	7	9.4	6.8	0.84	6.98	0.51	0.18	0.120
	10	8.9	77.7	4.23	7.36	0.53	0.17	0.170
	14	8.4	13.4	4.94	7.31	0.43	0.13	0.095
	19	7.9	20.8	5.39	7.30	N/D	N/D	lost
	23	7.4	41.1	6.82	8.05	N/D	N/D	0.165
	49	6.9	15.9	6.68	8.41	N/D	N/D	0.000
Alum	0	12	3.1	0.28	6.73	0	0.00	0.000
	1	11.4	2.9	0.10	6.97	0.02	0.02	0.000
	2	10.9	2.8	1.55	6.71	0.01	0.01	0.058
	3	10.4	9.5	0.62	6.83	0.08	0.06	0.180

Table B1 cont...

Table B1. (cont.)

Treatment	Day	Volume (L)	NO_3^- ($\mu g \cdot L^{-1}$)	TDP ($mg \cdot L^{-1}$)	pH	Exo-MCLR ($mg \cdot L^{-1}$)	Exo-MCLR ($mg \cdot g^{-1}$)	MCYST ($mg \cdot L^{-1}$)
Alum	4	9.9	58.5	0.19	6.88	0.07	0.05	0.044
	7	9.4	2.8	0.19	6.84	0.15	0.09	0.004
	10	8.9	8.4	0.79	7.32	0.16	0.09	0.006
	14	8.4	0.2	0.18	7.26	0.17	0.10	0.006
	19	7.9	5.7	0.11	7.48	N/D	N/D	lost
	23	7.4	14.4	0.37	8.03	N/D	N/D	0.007
	49	6.9	12.1	0.53	8.31	N/D	N/D	0.008
Control (15L)	0	12	2.4	0.05	6.79	0	0.00	0.000
	1	11.4	2.7	0.06	7.09	0	0.00	0.000
	2	10.9	N/D	1.44	6.84	0.04	0.05	0.026
	3	10.4	26.1	0.65	6.92	N/D	N/D	0.100
	4	9.9	92.9	0.23	7.04	0.03	0.03	0.006
	7	9.4	1.9	0.14	7.52	N/D	N/D	0.007
	10	8.9	7.2	1.69	7.63	N/D	N/D	0.007
	14	8.4	36.1	0.66	7.61	0.13	0.12	0.067
	19	7.9	5.2	0.12	7.68	N/D	N/D	lost
	23	7.4	16.5	0.69	8.22	N/D	N/D	0.005
	49	6.9	67.7	0.86	8.33	N/D	N/D	0.007
Lime	0	18	2.7	0.17	6.84	0.04	0.03	0.000
	1	17.4	5.0	2.51	6.77	0.07	0.05	0.080
	2	16.9	N/D	2.44	6.79	0.08	0.05	0.061
	3	16.4	17.2	1.81	6.80	0.08	0.05	0.083
	4	15.9	124.8	0.46	6.85	0.02	0.01	0.067
	7	14.9	5.8	1.61	7.04	0.07	0.04	0.046
	10	14.4	11.8	3.59	7.35	0.04	0.02	0.073
	14	13.9	97.7	2.07	7.31	0.04	0.02	0.078
	19	13.4	26.0	1.04	7.59	N/D	N/D	lost
	23	12.9	N/D	0.89	8.44	N/D	N/D	0.009
	49	12.4	42.2	1.92	8.63	N/D	N/D	0.009
Control (25L)	0	18	4.8	N/D	6.84	0.01	0.01	0.000
	1	17.4	5.2	2.12	6.82	0.01	0.01	0.042
	2	16.9	N/D	2.67	6.61	0.06	0.05	0.072
	3	16.4	107.8	2.12	6.66	0.07	0.05	0.024
	4	15.9	185.6	0.62	6.73	0.06	0.04	0.054
	7	14.9	8.6	1.55	6.87	0.18	0.12	0.033
	10	14.4	13.6	1.31	7.21	0.15	0.10	0.006
	14	13.9	85.6	1.56	7.19	N/D	N/D	0.006
	19	13.4	35.9	0.20	7.50	N/D	N/D	lost
	23	12.9	N/D	1.08	8.58	N/D	N/D	0.008
	49	12.4	69.1	0.27	8.63	N/D	N/D	0.009

Table B2. Average concentrations of dissolved microcystin-LR (exo-MCLR) and other chemical parameters in Batch 2. 'N/D' represents non-detectable, and 'N/A' represents not applicable.

Treatment	Day	Volume (L)	NO_3^- ($\mu g \cdot L^{-1}$)	NH_4^+ ($mg \cdot L^{-1}$)	TP ($mg \cdot L^{-1}$)	TDP ($mg \cdot L^{-1}$)	pH	Exo-MCLR ($mg \cdot L^{-1}$)	Exo-MCLR ($mg \cdot g^{-1}$)
Reglone A	0	11.5	3.4	0.41	0.69	0.61	8.46	0.001	0.001
	1	10.7	8.8	4.12	3.54	2.86	7.90	0.073	0.054
	2	9.9	9.4	11.27	3.63	3.35	7.53	0.259	0.221
	3	9.1	10.1	11.38	5.01	3.87	7.13	0.125	0.114
	4	8.3	7.6	18.51	5.70	4.12	7.17	0.003	0.003
	6	7.5	3.5	N/D	2.95	3.94	7.32	0.002	0.002
	9	6.7	16.5	20.87	4.70	3.89	7.43	N/D	N/D
	12	5.9	22.0	23.21	5.05	4.53	8.15	N/D	N/D
Alum	0	11.5	4.8	0.64	0.77	0.61	8.20	0.002	0.001
	1	10.7	5.8	3.08	0.62	0.13	7.25	0.081	0.067
	2	9.9	3.6	5.40	0.40	0.13	7.33	0.012	0.012
	3	9.1	4.9	8.04	0.86	0.11	7.33	0.001	0.001
	4	8.3	5.8	9.54	0.91	0.12	7.46	0.014	0.013
	6	7.5	6.6	N/D	0.32	0.12	7.76	0.005	0.004
	9	6.7	4.3	16.04	0.49	0.07	7.66	N/D	N/D
	12	5.9	9.6	20.64	0.71	0.29	7.66	N/D	N/D
$KMnO_4$	0	11.5	N/A	0.39	0.71	0.71	8.52	0.010	0.010
	1	10.7	N/A	5.47	1.24	0.86	7.33	0.034	0.033
	2	9.9	N/A	8.38	1.00	0.61	7.72	N/D	N/D
	3	9.1	N/A	10.35	0.61	0.54	7.52	0.002	0.002
	4	8.3	N/A	12.21	0.82	0.65	7.68	0.003	0.003
	6	7.5	N/A	N/D	1.22	0.95	7.90	0.005	0.005
	9	6.7	N/A	18.90	1.26	1.03	8.22	N/D	N/D
	12	5.9	N/A	20.86	1.61	1.51	8.31	N/D	N/D
Simazine	0	11.5	3.8	0.38	0.72	0.66	8.44	0.002	0.002
	1	10.7	6.2	5.71	1.24	0.86	7.29	0.085	0.087
	2	9.9	4.4	9.40	1.01	0.58	7.40	0.088	0.091
	3	9.1	5.6	14.15	0.71	0.60	7.42	0.092	0.096
	4	8.3	6.2	14.44	1.75	0.66	7.70	0.061	0.063
	6	7.5	2.3	N/D	1.19	0.84	7.90	0.064	0.067
	9	6.7	3.2	23.16	1.50	1.12	7.79	N/D	N/D
	12	5.9	24.2	26.01	1.93	1.64	8.07	N/D	N/D
NaOCl	0	11.5	3.3	0.38	0.66	0.61	8.28	0.003	0.003
	1	10.7	12.8	2.23	N/D	1.74	0.00	0.198	0.212
	2	9.9	9.2	9.40	2.55	1.42	7.30	0.014	0.015
	3	9.1	2.2	6.73	2.92	1.34	7.18	0.041	0.044
	4	8.3	1.1	12.96	3.31	1.57	7.71	0.005	0.006

Table B2 cont...

Table B2. (cont.)

Treatment	Day	Volume (L)	NO_3^- ($\mu g \cdot L^{-1}$)	NH_4^+ ($mg \cdot L^{-1}$)	TP ($mg \cdot L^{-1}$)	TDP ($mg \cdot L^{-1}$)	pH	Exo-MCLR ($mg \cdot L^{-1}$)	($mg \cdot g^{-1}$)
NaOCl	6	7.5	2.4	N/D	3.11	0.78	7.49	N/D	N/D
	9	6.7	7.9	21.24	2.72	1.38	7.54	N/D	N/D
	12	5.9	8.6	22.03	3.01	2.11	7.98	N/D	N/D
Lime	0	11.5	4.0	0.55	0.70	0.63	8.29	0.002	0.002
	1	10.7	5.9	6.54	1.06	0.72	7.43	0.011	0.010
	2	9.9	5.2	10.05	0.68	0.43	7.43	0.008	0.007
	3	9.1	6.5	9.89	0.92	0.50	7.49	0.004	0.003
	4	8.3	6.1	13.42	1.14	0.58	7.60	0.002	0.002
	6	7.5	6.9	N/D	0.55	0.83	7.96	0.003	0.003
	9	6.7	2.3	20.08	1.34	1.09	8.14	N/D	N/D
	12	5.9	19.2	21.25	1.72	1.56	8.41	N/D	N/D
Control	0	11.5	2.4	0.27	0.69	0.61	8.54	0.001	0.001
	1	10.7	5.7	5.49	1.12	0.77	7.71	0.002	0.002
	2	9.9	4.3	7.86	0.89	0.46	7.40	0.007	0.007
	3	9.1	4.9	12.53	1.29	0.49	7.50	0.014	0.011
	4	8.3	11.1	13.28	1.51	0.73	7.66	0.008	0.007
	6	7.5	4.8	N/D	1.39	0.93	7.98	0.006	0.006
	9	6.7	8.9	19.52	1.26	1.08	8.17	N/D	N/D
	12	5.9	N/D	20.96	2.02	1.59	8.13	N/D	N/D

Table B3. Average concentrations of dissolved microcystin-LR (exo-MCLR) and other chemical parameters in Batch 3. 'N/D' represents non-detectable, and 'N/A' represents not applicable.

Treatment	Day	Volume (L)	NH_4^+ ($mg \cdot L^{-1}$)	TP ($mg \cdot L^{-1}$)	DOC ($mg \cdot L^{-1}$)	pH	Exo-MCLR ($mg \cdot L^{-1}$)	Exo-MCLR ($mg \cdot g^{-1}$)
Reglone A	0	11	0.12	0.82	22	8.37	0.001	0.001
	2hr	11	N/A	N/A	N/A	7.89	N/A	N/A
	1	10.2	3.40	3.48	39	7.48	0.053	0.058
	2	9.4	8.63	4.78	44	7.51	0.115	0.126
	3	8.6	14.06	6.19	57	7.52	0.031	0.033
	5	7.8	23.26	6.61	61	7.79	0.027	0.029
	8	7	53.23	8.38	65	7.95	0.029	0.031
	12	6.2	35.21	8.80	60	8.52	0.030	0.033
Alum	0	11	0.08	0.79	22	8.36	0.001	0.002
	2hr	11	N/A	N/A	N/A	6.60	N/A	N/A
	1	10.2	0.41	0.60	23	6.72	0.022	0.025
	2	9.4	2.87	0.49	25	7.00	0.037	0.044
	3	8.6	14.06	6.19	57	7.21	0.016	0.019
	5	7.8	12.78	0.19	23	7.38	0.010	0.011
	8	7	39.92	0.30	27	7.65	N/D	N/D
	12	6.2	32.36	0.58	N/D	7.94	N/D	N/D
$KMnO_4$ (10 $mg \cdot L^{-1}$)	0	11	0.15	0.81	22	8.47	0.001	0.002
	2hr	11	N/A	N/A	N/A	7.85	N/A	N/A
	1	10.2	4.81	2.44	31	7.39	0.037	0.043
	2	9.4	8.45	1.91	32	7.68	N/D	N/D
	3	8.6	11.91	2.04	33	7.77	N/D	N/D
	5	7.8	18.79	2.21	33	7.91	N/D	N/D
	8	7	41.08	2.96	27	8.27	N/D	N/D
	12	6.2	24.41	3.02	27	8.51	N/D	N/D
$KMnO_4$ (12.5 $mg \cdot L^{-1}$)	0	11	0.13	0.88	22	8.34	0.003	0.004
	2hr	11	N/A	N/A	N/A	7.72	N/A	N/A
	1	10.2	5.08	2.36	30	7.46	0.031	0.033
	2	9.4	9.62	2.01	38	7.59	0.002	0.003
	3	8.6	14.62	2.50	31	7.76	N/D	N/D
	5	7.8	20.35	2.93	32	7.92	N/D	N/D
	8	7	46.37	4.05	36	8.33	N/D	N/D
	12	6.2	30.62	3.16	28	8.43	N/D	N/D
NaOCl	0	11	0.12	0.81	22	8.38	0.002	0.002
	2hr	11	N/A	N/A	N/A	8.04	N/A	N/A
	1	10.2	5.08	3.69	40	7.56	0.150	0.163
	2	9.4	7.95	3.71	41	7.50	0.028	0.031

Table 3B cont...

Table 3B. (Cont.)

Treatment	Day	Volume (L)	NH_4^+ ($mg \cdot L^{-1}$)	TP ($mg \cdot L^{-1}$)	DOC ($mg \cdot L^{-1}$)	pH	Exo-MCLR ($mg \cdot L^{-1}$)	Exo-MCLR ($mg \cdot g^{-1}$ algae)
NaOCl	3	8.6	11.27	3.69	37	7.67	N/D	N/D
	5	7.8	19.72	4.09	41	7.85	N/D	N/D
	8	7	43.84	4.86	40	8.05	N/D	N/D
	12	6.2	30.41	4.67	35	8.22	N/D	N/D
Lime	0	11	0.10	0.79	25	8.30	0.002	0.002
	2hr	11	N/A	N/A	N/A	10.60	N/A	N/A
	1	10.2	3.55	1.05	25	9.99	0.000	0.000
	2	9.4	11.66	1.32	28	8.51	0.022	0.021
	3	8.6	16.51	1.86	28	7.96	0.004	0.004
	5	7.8	26.73	2.76	33	7.99	0.001	0.000
	8	7	56.81	3.84	35	8.35	0.001	0.001
	12	6.2	37.47	4.11	30	8.44	N/D	N/D
	19	5.4	9.84	4.31	30	7.85	N/D	N/D
	23	4.6	0.07	4.05	30	0.00	N/D	N/D
Control	0	11	0.16	0.88	25	8.24	0.001	0.001
	2hr	11	N/A	N/A	N/A	7.91	N/A	N/A
	1	10.2	4.56	1.71	29	7.39	0.009	0.011
	2	9.4	6.68	1.39	28	7.73	N/D	N/D
	3	8.6	9.43	1.30	27	7.81	0.002	0.002
	5	7.8	16.96	1.85	28	7.97	N/D	N/D
	8	7	38.90	2.41	25	8.29	N/D	N/D
	12	6.2	19.01	2.78	25	8.12	N/D	N/D
	19	5.4	0.02	3.19	27	8.22	N/D	N/D
	23	4.6	0.01	4.10	28	0.00	N/D	N/D

Table 3C. Bacterial density ($CFU \cdot mL^{-1}$) estimated from Starch Casein + Cycloheximide Agar before (day 0) and after (day 7) chemical addition in Batch 1.

Treatment	Bacterial density ($CFU \cdot mL^{-1}$)	
	day 0	day 7
Reglone A	1.2×10^6	9×10^5
Alum	8.7×10^5	1×10^5
$KMnO_4$	7.4×10^5	3.8×10^5
Simazine	9.7×10^5	1×10^6
Control (15L)	8.9×10^5	7.4×10^3
Lime	4.7×10^5	1.2×10^5
Control (25L)	1×10^5	3×10^4

XI. Appendix C:

Data for the sorption and limnocorral experiments

Table C1. Concentration of MCLR remained in the water phase of the sorption experiment.

Treatment	MCLR remained ($\mu g \cdot mL^{-1}$)			MCLR remained (%)		
	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
Control #1	0.11	0.08	0.08	113	89	92
Control #2	0.08	0.10	0.10	87	116	115
Control #3	0.10	0.08	0.08	100	95	94
Mean	**0.10**					
5 $g \cdot L^{-1}$ #1	0.12	0.09	0.11	104	83	100
5 $g \cdot L^{-1}$ #2	0.12	0.10	0.11	101	91	105
5 $g \cdot L^{-1}$ #3	0.11	0.13	0.10	95	126	95
Mean	**0.12**					
10 $g \cdot L^{-1}$ #1	0.11	0.12	0.07	96	113	72
10 $g \cdot L^{-1}$ #2	0.11	0.09	0.12	91	84	118
10 $g \cdot L^{-1}$ #3	0.13	0.11	0.11	114	103	110
Mean	**0.12**					

Table C2. A summary of the characteristics of all the limnocorrals and the reference site for Experiment 1 over the 4-day period. Phytoplankton abundance are expressed in dry weight and cell volume, *Microcystis aeruginosa* is expressed as cell volume, and dissolved microcystin concentration (Exo-MYST) is expressed as microcystin-LR equivalent per liter lake water. Chemical parameters such as ammonium (NH_4^+), nitrate+nitrite (NO_3^-), total phosphorus (TP), total dissolved phosphorus (TDP), and pH are also included.

Treatment	Day	Phytoplankton		*Microcystis*	Exo-MYST	NH_4^+	NO_3^-	TP	TDP	pH
		drywt $mg \cdot L^{-1}$	$mm^3 \cdot L^{-1}$	$mm^3 \cdot L^{-1}$	$\mu g \cdot L^{-1}$	$\mu g \cdot L^{-1}$	$\mu g \cdot L^{-1}$	$\mu g \cdot L^{-1}$	$\mu g \cdot L^{-1}$	
Control	0	0.76	15.54	0.52	N/D	9.7	7.2	305	232	9.3
	1	0.82	28.74	1.21	0.030	19.1	2.3	300	224	9.5
	2	0.78	18.23	0.71	0.035	13.2	3.4	353	258	9.1
	3	1.33	10.88	3.25	0.040	61.5	28.8	503	420	9.2
	4	N/D	17.23	2.36	0.040	26.7	4	496	379	9.7
Reference	0	1.23	14.21	2.86	0.045	7.3	9.6	315	268	9.2
	1	0.78	16.43	1.98	0.015	27	7.2	356	308	9.4
	2	1.40	8.31	2.77	0.055	77.9	14.2	493	414	8.8
	3	1.11	13.23	2.68	0.160	80.9	26.1	504	408	9.5
	4	0.75	28.46	1.53	0.075	23.5	11.5	439	368	9.7
Lime	0	0.61	19.04	0.20	0.055	6.5	7.9	314	243	9.2
	1	0.15	2.75	0.14	0.260	32.4	3.5	101	50	10.4
	2	0.13	1.94	0.20	0.320	110.7	7.5	118	64	9.8
	3	0.57	4.42	0.61	0.260	107.9	9.1	193	127	10.2
	4	0.86	14.16	1.30	0.165	57.3	10.9	240	167	10.2
Reglone A	0	0.56	18.99	0.33	N/D	7.7	8.6	293	239	9.2
	1	0.19	5.66	2.16	0.195	70	5.8	410	367	9.3
	2	0.09	6.95	2.52	0.405	136.6	6.2	429	393	8.8
	3	0.13	6.21	3.58	0.415	101.1	11.8	457	407	9.0
	4	0.47	11.41	3.38	0.425	121.8	12.5	476	425	9.5

Table C3. A summary of phytoplankton biomass (expressed as algal dry-weight per liter lake water) and both intracellular microcystin (Endo-MYST) and dissolved microcystin (Exo-MYST) concentrations (expressed as µg equivalent of microcystin-LR per liter lake water; PP bioassay) for all limnocorrals and reference site of Experiment 2 over the 7-day period. Intracellular microcystin-LR concentration (Endo-MCLR) at day 0 was also measured by HPLC. 'N/D' = not detectable, and 'N/S' = no sample.

Treatment	Day	Phytoplankton drywt. $mg \cdot L^{-1}$	Endo-MYST $\mu g \cdot mg^{-1}$ algae	Endo-MYST $\mu g \cdot L^{-1}$	Exo-MYST $\mu g \cdot L^{-1}$	Endo-MCLR $\mu g \cdot mg^{-1}$ algae
Control 1	0	0.36	0.54	0.19	0.19	0.18
	1	0.54	0.71	0.38	N/D	-
	2	0.54	0.62	0.34	N/D	-
	3	0.73	0.68	0.50	N/D	-
	4	0.49	0.53	0.26	N/D	-
	7	0.97	0.81	0.78	N/D	-
Control 2	0	1.90	0.41	0.78	0.31	0.14
	1	1.94	0.33	0.64	N/D	-
	2	2.23	0.22	0.50	N/D	-
	3	2.19	0.25	0.54	N/D	-
	4	1.88	0.12	0.23	N/D	-
	7	2.91	0.13	0.37	0.15	-
Control 3	0	2.06	0.48	1.00	0.38	0.14
	1	1.93	0.22	0.43	N/D	-
	2	2.05	0.84	1.72	N/D	-
	3	1.86	0.12	0.22	N/D	-
	4	2.10	0.12	0.25	N/D	-
	7	2.87	0.51	1.46	N/D	-
Reference 1	0	1.10	1.59	1.76	0.33	0.72
	1	1.08	1.17	1.27	0.16	-
	2	2.17	0.94	2.05	N/D	-
	3	1.65	0.78	1.28	0.07	-
	4	1.72	0.72	1.25	N/D	-
	7	2.33	0.59	1.38	0.07	-
Reference 2	0	1.26	1.34	1.69	0.29	0.65
	1	1.03	1.24	1.28	N/D	-
	2	1.48	1.03	1.53	N/D	-
	3	2.03	0.81	1.63	0.07	-
	4	2.04	0.61	1.24	N/D	-
	7	2.48	0.76	1.90	N/D	-
Reference 3	0	0.81	1.37	1.11	0.14	0.70
	1	1.01	1.29	1.31	N/D	-

Table C2 cont...

Table C3. (cont.)

Treatment	Day	Phytoplankton drywt. $mg \cdot L^{-1}$	Endo-MYST $\mu g \cdot mg^{-1}$ algae	Endo-MYST $\mu g \cdot L^{-1}$	Exo-MYST $\mu g \cdot L^{-1}$	Endo-MCLR $\mu g \cdot mg^{-1}$ algae
Reference 3	2	2.47	0.78	1.91	N/D	-
	3	2.25	0.84	1.90	0.34	-
	4	2.02	0.72	1.45	N/D	-
	7	2.54	0.48	1.23	N/D	-
Lime/Alum 1	0	0.74	0.44	0.32	0.33	0.14
	1	0.09	0.12	0.01	N/D	-
	2	0.05	0.58	0.03	N/D	-
	3	0.08	0.27	0.02	N/D	-
	4	0.07	0.26	0.02	N/D	-
	7	0.11	0.45	0.05	0.07	-
Lime/Alum 2	0	N/S	N/S	N/S	0.33	N/S
	1	0.22	0.20	0.04	N/D	-
	2	0.10	0.09	0.01	0.16	-
	3	0.14	0.06	0.01	N/D	-
	4	0.09	0.08	0.01	N/D	-
	7	1.12	0.90	1.00	N/D	-
Lime/Alum 3	0	2.01	0.57	1.14	0.34	0.13
	1	0.20	0.12	0.02	0.15	-
	2	0.10	0.77	0.07	0.18	-
	3	0.20	0.08	0.02	0.29	-
	4	0.21	0.11	0.02	N/D	-
	7	0.19	0.45	0.08	N/D	-
Reglone 1	0	1.38	0.48	0.67	0.35	0.18
	1	0.32	2.07	0.66	0.65	-
	2	0.35	2.25	0.79	3.55	-
	3	0.27	0.92	0.25	3.35	-
	4	0.09	0.36	0.03	2.35	-
	7	0.06	0.61	0.04	3.25	-
Reglone 2	0	2.96	0.59	1.75	0.33	0.23
	1	1.30	1.31	1.69	1.65	-
	2	0.78	0.83	0.65	3.65	-
	3	0.26	N/S	N/S	3.45	-
	4	0.29	0.10	0.03	4.00	-
	7	0.71	0.63	0.45	3.35	-
Reglone 3	0	1.83	0.47	0.87	0.30	0.15
	1	0.55	1.98	1.09	1.73	-
	2	0.39	1.43	0.56	3.85	-
	3	0.26	0.29	0.08	3.25	-
	4	0.11	0.20	0.02	2.20	-
	7	0.45	0.21	0.10	3.50	-

Table C4. Summary of the nutrient characteristics of all the limnocorrals and the reference site for Experiment 2 over the 7-day period. Note that TP = total phosphorus; TDP = total dissolved phosphorus; Chl*a* = chlorophyll *a*; NH_4^+ = ammonium; NO_3^- = nitrate+nitrite; and K = potassium concentration.

Treatment	Day	TP ($\mu g \cdot L^{-1}$)		TDP ($\mu g \cdot L^{-1}$)		Chl*a* ($\mu g \cdot L^{-1}$)	
		Mean	*SE*	Mean	*SE*	Mean	*SE*
Control	0	638	18	538	20	65	19
	1	627	22	522	24	64	20
	2	652	13	522	12	71	26
	3	656	49	503	7	50	17
	4	669	28	504	20	70	30
	7	650	19	483	29	74	34
Reference	0	663	17	582	10	33	1
	1	684	10	611	3	30	0
	2	697	7	608	2	39	5
	3	705	7	608	14	52	3
	4	752	10	652	2	54	1
	7	815	9	735	29	76	2
Lime/Alum	0	661	38	379	6	69	15
	1	114	4	45	3	4	1
	2	99	5	39	3	2	0
	3	94	1	41	3	3	0
	4	117	8	49	7	5	1
	7	330	194	231	183	17	8
Reglone A	0	675	15	524	17	91	20
	1	770	13	687	6	42	8
	2	778	42	688	16	26	5
	3	820	32	720	22	11	1
	4	824	14	764	15	7	1
	7	878	30	779	13	4	2

Table C4 cont...

Table C4 (cont.)

Treatment	Day	NH_4^+ ($\mu g \cdot L^{-1}$) Mean	*SE*	NO_3^- ($\mu g \cdot L^{-1}$) Mean	*SE*	K ($mg \cdot L^{-1}$) Mean	*SE*
Control	0	36	24	4	3	9.15	0.28
	1	34	3	1	0	9.09	0.22
	2	33	17	2	0	9.50	0.02
	3	284	132	2	0	9.50	0.08
	4	89	47	2	1	9.49	0.08
	7	21	1	0	0	9.25	0.15
Reference	0	2	2	3	1	9.04	0.11
	1	35	2	2	1	9.18	0.12
	2	0	0	1	0	9.57	0.08
	3	0	0	0	0	9.69	0.11
	4	0	0	1	0	9.49	0.04
	7	19	1	0	0	9.30	0.11
Lime/Alum	0	15	1	3	1	9.19	0.09
	1	52	7	4	1	9.28	0.10
	2	82	3	6	1	9.76	0.04
	3	115	18	2	0	9.93	0.11
	4	100	33	2	0	9.78	0.04
	7	41	12	0	0	9.64	0.12
Reglone A	0	20	11	3	2	9.25	0.15
	1	108	4	7	1	9.57	0.17
	2	150	16	3	1	9.77	0.02
	3	245	95	2	0	10.36	0.20
	4	212	23	4	1	10.57	0.13
	7	288	23	1	0	10.36	0.14

X. Appendix D:

Bacterial densities and toxin data for the biotransformation Experiments 1 and 2

Table D1. Bacterial density ($CFU \cdot mL^{-1}$) estimated from Plate Count Agar in Experiment 1. 'N/S' indicates no sample.

	Day	Replicates A	B	C
Control	0	0	0	0
	3	0	0	0
	7	0	0	0
	14	0	0	0
	21	0	0	0
	28	N/S	N/S	N/S
Culture	0	29,900	44,950	34,800
	3	171,400	429,000	500,000
	7	186,000	86,700	180,300
	14	21,785	11,550	142,700
	21	17,600	22,170	39,130
	28	N/S	N/S	N/S
Reference	0	28,150	36,850	45,000
	3	471,000	589,350	353,000
	7	151,300	175,700	72,850
	14	37,815	35,785	50,970
	21	15,800	19,670	127,215
	28	N/S	N/S	N/S

Table D2. Bacterial density ($CFU \cdot mL^{-1}$) estimated from Plate Count Agar in Experiment 2.

Day	Control	Culture A	Culture B
0	0	57,900	59,333
3	0	753,333	396,667
7	0	157,667	103,000
14	0	295,667	116,000
16	0	836,667	93,000

Table D3. Microcystin-LR concentration ($\mu g \cdot L^{-1}$; HPLC) in Experiment 1. 'Lost' indicates missing sample, and 'N/D' indicates non-detectable.

	Day	Replicates A	B	C	Mean	*SE*
Control	0	227	214	234	225	6
	3	222	204	214	214	5
	7	193	187	198	193	3
	14	212	201	203	205	4
	21	202	178	207	196	9
	28	Lost	241	241	161	80
Culture	0	247	236	238	241	3
	3	216	220	206	214	4
	7	198	200	194	197	2
	14	73	73	74	74	0
	21	17	18	8	14	3
	28	N/D	N/D	N/D	N/D	N/D

Table D4. Concentrations of microcystin-LR ($\mu g \cdot L^{-1}$; HPLC) and total microcystin (μg MCLR equivalent per liter; PP bioassay) in Experiment 2. 'N/D' indicates non-detectable.

Day	HPLC analysis Control	Culture A	Culture B	PP bioassay Control	Culture A	Culture B
0	578	552	484	321	277	281
3	595	608	518	378	602	402
7	562	558	488	285	386	353
10	563	553	483	434	378	450
14	596	56	51	542	0.6	0.9
16	612	N/D	N/D	723	0.5	0.5
After 2nd MCLR spiking						
16	1230	1622	717	1205	1205	590
16.33	1299	397	671	1277	361	578
16.75	1284	N/D	585	1277	2.5	563
17.10	1264	N/D	165	1301	1.4	54
17.90	1219	N/D	N/D	1277	1.6	1.8
18.90	1261	N/D	N/D	1325	1.3	0.8
19.77	1259	N/D	N/D	1277	1.2	1.8